THE TROPHIC ECOLOGY OF WATERBIRDS IN A SMALL TEMPERATE ESTUARY: A STABLE ISOTOPE AND LIPID TRACER APPROACH.

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JEFFREY WILLIAM HEAN

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Abstract

Waterbirds are often overlooked as predators in aquatic ecosystems, despite the fact that waterbirds congregate in great numbers in and around waterways, notably estuaries. To fully appreciate the effect that aquatic feeding waterbird species may have on aquatic prey communities and the role that they play in estuarine food webs, stable isotopes and fatty acid profiles were employed to examine the seasonal diet of selected waterbirds in the Kowie Estuary, Eastern Cape Province, South Africa. Population counts were conducted every month for four seasons to examine the demography of waterbirds in the lower reaches of the estuary. The mean monthly energy consumption, along with dry matter intake of all waterbird species observed, were calculated and compared to similar estuaries in South Africa and elsewhere. Three duck species, one migrant sandpiper and one piscivore were selected for more detailed investigation at several temporal scales. This thesis has revealed that stable isotope analysis of waterbird tissues provides more informative data than fatty acid analysis for investigating waterbird diet and basal resource-tertiary consumer nutrient coupling. Stable $\delta^{15}N$ and $\delta^{13}C$ isotopes from several body tissues, in conjunction with SIAR models, were used to determine the seasonal diet of each waterbird species, while fatty acid profiles were investigated to examine the trophic transfer of fatty acids from basal resources to waterbird predators via the benthic fauna. Stable isotopes revealed that Cape Shoveller, Cape Teal and Yellow-Billed Duck shifted their diet over both long and short temporal scales, while the migratory Ruff and piscivorous Little Egret maintained a relatively consistent diet over time. Isopods, amphipods, copepods and Mysidacea were the main foods of all three duck species and the Ruff (>30%). Little Egret fed mainly on flathead mullet throughout the year. Fatty acid analysis revealed evidence for trophic transfer of specific fatty acids from basal resources to waterbirds in the Kowie Estuary but provided little information on seasonal diet of waterbirds. Waterbirds foraging in the Kowie Estuary appeared to shift their diet to coincide with resource abundance pulses, but also displayed seasonal dietary overlap. This study highlights the role that waterbirds play in aquatic food webs. The subject requires more attention so that we can better understand all the predatory drivers on aquatic communities.

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Table Headings

To my parents, Hamish and Louise Hean: With all my love, this is for you!

"*Vi veri Universum vivus vici* - By the power of truth, I, while living, have conquered the Universe"

-Aleister Crowley

"There are two important days in your life – the day you are born and the day you figure out why"

-Unknown

Chapter 1: Introduction and Overview

Determination of energy flow pathways in food webs is necessary to assess the structure and functional trophic roles of the organisms inhabiting ecosystems (Pasquad et al. 2010). Aquatic-terrestrial ecotones are prime examples of discontinuities between physically well-bounded ecosystems (Post et al. 2007b). Material fluxes transcend the land–water boundary in numerous settings, and cross-boundary transfers substantially modify ecological processes in lakes (Pace et al. 2007), streams (Burdon and Harding 2007) wetlands (Rubbo et al. 2006), estuaries (Chanton and Lewis 2002), and marine shorelines (Polis and Hurd 1996). Theoretical frameworks of meta-ecosystems incorporate spatial connectivity integrate perspectives of community and landscape ecology and provide a useful conceptualisation of ecosystem functioning, dynamics and stability (Loreau et al. 2003). The flow of nutrients and organic matter from one habitat to another links production and consumption processes, and recipient ecosystems typically become energetically connected to donor systems via trophic subsidies (Polis et al. 1997a). Trophic subsidies can strongly influence population dynamics, predator–prey interactions and food webs (Loreau and Holt 2004), while ecologists are increasingly recognizing their fundamental structuring role (Marczak et al. 2007). Significant effects of subsidies span a wide range of ecological organization, including stimulating primary productivity (Schlachter et al. 2008), vegetation structure modification (Ellis et al. 2006), increases in the abundance and biomass of consumers (Stapp and Polis 2003, Paetzold et al. 2006, Marczak et al. 2007), modulations of predator–prey interactions (Knight et al. 2005, Schlachter and Cronin 2007), alterations of the dynamics of spatially-coupled food webs (McCann et al. 2005), and shifts in ecosystem metabolism (Rubbo et al. 2006, Pace et al. 2007).

1.1 River and Estuarine ecosystems

Food web analysis has been used to examine the structure, dynamics and overall functioning of rivers and estuaries throughout the world (Garcia et al. 2007, Pasquaud et al. 2008). Estuaries have long been recognized as some of the most productive natural ecosystems in the world because they play a crucial role in maintaining biodiversity, particularly for fish (Constanza et al. 1997). The high production of diverse primary producers, together with the transportation of organic matter from adjacent rivers and marine environs supports estuarine food webs (McLuskv 1989, Choy et al. 2009).

Rivers and their associated estuaries are spatially complex ecosystems that provide a number of key ecological functions (França et al. 2011); 1) they support high fish and invertebrate species diversity and abundance, 2) they provide nursery areas for numerous marine fish species, 3) they fulfil the role of conduits for species which move between ocean and rivers (e.g. eel, salmon, trout, invertebrates, etc) and 4) they serve as staging sites for significant populations of migratory birds (Turpie 1995, Jennings and Warr 2003, Alfaro et al. 2006, França et al. 2011). Rivers and estuaries support a number of endemic bird species, many of which depend on estuaries for their survival. However, despite being classified as biological hotspots, South African estuaries constitute some of the most threatened habitats in the country (Turpie et al. 2002). At present, there are approximately 250 functional rivers and estuaries along the South African coastline (Whitfield, 2000), encompassing an area of approximately 70 000 ha (Turpie et al. 2002). Over the past several decades, there have been increasing numbers of incidents of human disturbance such as upstream water extraction, weir creation, pollution and agricultural run-off, exploitation of natural resources and resort developments along South African rivers and estuaries, all resulting in disruptions in freshwater inflows, sediment characteristics or nutrient supplies to estuaries (Turpie et al. 2002). Many South African river systems and estuaries have therefore become functionally degraded, often accompanied by a loss of species (e.g. Goliath Heron, *Ardea goliath*, from the Swartkops River and Estuarine Pipefish, *Sygnathus watermeyeri*, from the Kariega River; Whitfield 1998) or reductions in population abundances (Whitfield 1998, Woolridge 1999). Despite the conservation emphasis that is placed on rivers and estuaries in South Africa, we lack critical information pertaining to the general ecology and food web dynamics of riverine and estuarine ecosystems.

Conventional models of aquatic food webs assume that fish are the most important biotic factors that influence organisms occupying lower trophic levels, as fish are presumed to fill the apex trophic level (Vannote et al. 1980, Fry 1991, Wellborn et al. 1996). However, many terrestrial predators, including many bird species, feed in aquatic systems, and therefore are influential components of aquatic food webs (Steinmetz et al. 2003). They often fill the niche of predators (e.g. piscivorous birds) and exert top-down pressure on lower trophic levels. Aquatic-feeding waterbirds have a significant predatory impact on the community of invertebrates and remove a substantial proportion of the annual production of benthic macrofauna (e.g. Szekely and Bamberger 1992, Turpie and Hockey 1996, Hockey and Turpie 1999, Kober et al. 2006, Lourenc et al. 2008, Kaletjta-Summers et al. 2009, Froneman et al. 2011). South African rivers and estuaries provide refuge to several hundred bird species that fill a wide range of ecological niches (Day 1981).

The items that waterbirds feed upon are diverse, ranging from aquatic vegetation and algae, to invertebrates (molluscs, crustaceans, polychaetes, annelids) and vertebrates (amphibians and fish). Waterbirds play pivotal roles in nutrient cycling, maintenance of prey populations, and dispersal of plant seeds in some instances (Day 1981). The net energy consumption of shorebirds in the Riet River (including the estuary) in South Africa, was estimated at 45-52% of total energy consumption in the ecosystem, with invertebrate feeders responsible for 49% of the total consumption (Kaletjta-Summers et al. 2009). Similarly, invertebrate-feeding wading birds were calculated to consume 26% ±10% of the net annual invertebrate production in the Berg Estuary, South Africa (Kalejta 1992). Thus, bird species that feed in estuarine habitats play an important role in energy fluxes across estuarine food webs (Moreira 1997b). There is a plethora of information on trophic subsides between seabirds and their marine prey (e.g. Cherel et al. 2005, Karnovsky et al. 2008, Hebert et al. 2009, Jaeger et al. 2009, 2010, Richoux et al. 2010, Wold et al. 2011), with global seabird consumption rivalling that of annual global fisheries catches (Brooke 2004). Surprisingly, little is known regarding the ecological role of waterbirds in estuarine and freshwater relative to marine systems. How avian consumers use adjacent habitats or ecosystems to obtain their daily dietary requirements is imperative to determine if we are to elucidate how avian consumers contribute to and influence aquatic food webs.

1.2 Stable isotope and fatty acid tracers in food web studies

Elucidation of food web dynamics and ecosystem community structure has conventionally been based on gut content analyses (particularly in large consumers) and faecal analysis, supported by direct field observations (Edgar 1990, Alfaro et al. 2006). Although a significant amount of information can be obtained through gut-content and faecal analysis, these methods are time-consuming and logistically difficult (Hanson et al. 2010), while gut-content analysis is intrusive and destructive to the organism concerned. In addition, differentiating between which items in the gut have been ingested and those that are actually assimilated can also be difficult to ascertain (Hanson et al. 2010). Although much of our current understanding of predator diets is derived from these methods, such estimates can be biased (Jobling and Breiby 1986, Jobling 1987, Carss and Parkinson 2009). Soft-bodied prey items are difficult to identify, given that they are rapidly digested (Iverson et al. 2004), while hardened body parts, such as shells, exoskeletons and bone fragments, will remain in the gut for longer periods of time. Retention of these types of body parts will often lead to bias in that the size of prey items consumed may be underestimated or the identification of prey may not be possible (Iverson et al. 2004).

Furthermore, the degree of erosion of hard parts is species specific and often a function of prey size within species (Bowen 2000). Inevitably, differential rates of digestion among prey species will seriously bias estimates of consumer diet in favour of species with large and robust hard parts (Iverson et al. 2004). The use of intrinsic tracers such as stable isotopes and lipids, has been favoured by ecologists over the past three decades. Stable isotope ratios and fatty acid profiles provide details on the sources of nutrition assimilated over long periods of time, rather than merely recent ingestion (Dalsgaard et al. 2003, Michener and Schell 2007). These tracers are affected by biases related to metabolic processes in consumers, and natural variation in or similarities among dietary sources (Hobson and Clark 1992, Gladyshev et al. 2011, Syväranta et al. 2013). Despite these caveats, stable isotopes and fatty acid profiles have been successfully used to determine consumer diets (Käkelä et al. 2006, 2007, 2010, Alfaro et al. 2006), describe inter- and intraspecific consumer trophodynamics (Berlow et al. 1999, Awkerman et al. 2007, Cherel et al. 2008a, Hebert et al. 2009b, Christensen and Moore 2009, Hopkins and Ferguson 2012), and investigate trophic position shifts and cascades (Post 2002, Budge et al. 2007, Cherel et al. 2007, Quevedo et al. 2009, Young et al. 2010, Fort et al. 2010, Doucette et al. 2011).

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are two commonly used stable isotopes in the study of food web dynamics (Abrantes and Sheaves 2010), but $\delta^{18}O$, $\delta^{2}H$, and $\delta^{34}S$ have also been used with success (e.g. Hobson and Wassenaar 1997, Soto et al. 2013). Nitrogen and carbon stable isotope ratios in consumers are typically enriched from their prey by 3.4‰ and 1‰ respectively (Deniro and Epstein 1981). However, numerous studies have revealed that $\delta^{15}N$ and $\delta^{13}C$ trophic enrichment values are highly dependent on the type of consumer being studied (e.g. Post 2002a, Caut et al. 2009, Boecklen et al. 2011) and even species-specific in some instances (e.g. Vander Zanden and Rasmussen 1999, Matthews and Mazumder 2004). Factors that primarily account for this stochasticity include the quality of dietary protein, metabolic processes, growth rate, age and size of the consumer, tissue type being sampled and tissue sampling technique (Vanderklift and Ponsard 2003, Caut et al. 2009, Perkins et al. 2014), although there is much debate surrounding which elements are most important (Perkins et al. 2014). Nevertheless, despite these uncertainties, $\delta^{13}C$ can be used to trace the origin of carbon sources for a consumer (Michener and Kaufman 2007). Because ecosystems can exhibit strikingly disparate stable isotope ratios, stable isotopes can also be used to trace carbon sources of consumers that originate in adjacent ecosystems (e.g. aquatic vs. terrestrial, nearshore marine vs. offshore marine; Rounick and Winterbourn 1986). The larger $\delta^{15}N$ shift between a consumer and its prey means that $\delta^{15}N$ signatures can be reliable indicators of the trophic position of an organism within the food web relative to the primary producers (Pasquad et al. 2010).

Relative ^{15}N enrichment indicates the trophic level of an organism – $\delta^{15}N$ values in consumers are high if the consumer is a strict predator, primary/secondary consumers have $\delta^{15}N$ values at the relatively intermediate level, while herbivores exhibit the lowest $\delta^{15}N$ values compared to other consumers (Kling et al. 1992). The exclusive use of $\delta^{15}N$ to calculate trophic levels can lead to erroneous results because primary producers in the same area can have varying $\delta^{15}N$ signatures, and $\delta^{13}C$ and $\delta^{15}N$ values of primary producers may not be independent of one another (Hobson and Clark 1992a,b, Angradi 1994, France 1997, Post 2002, Post et al. 2007a). If the potential sources cover a wide range in $\delta^{13}C$, the exact $\delta^{15}N$ level representing a particular trophic level will vary depending on $\delta^{13}C$ (Post 2002).

It is therefore preferable to use both $\delta^{15}N$ and $\delta^{13}C$ values to accurately analyse trophic levels in food webs (Vander Zanden and Rasmussen 1999, Post 2002b, Post et al. 2007b). Despite being methodologically complex, specific fatty acids can be traced from primary producers through to consumers at several trophic levels (Iverson et al. 2004b, Iverson 2008, Pollierer et al. 2010). Fatty acids are therefore particularly useful for investigating the feeding ecology of consumers with broad diet ranges (Ramos and González-Solís 2012), such as birds, which are traditionally logistically difficult to study (Käkelä et al. 2009, Williams and Buck 2010, Karnovsky et al. 2012). Lipid components that are physiologically important to animals, such as highly unsaturated fatty acids (HUFAs), are relatively scarce in terrestrial ecosystems, but are abundant in aquatic ecosystems (Gladyshev et al. 2009, 2011). Although terrestrial plants are able to produce some biologically important fatty acids, they cannot desaturate and elongate the most essential components that many algal species are able to (Gladyshev et al. 2011). Most higher consumers can auto-synthesise some lipid components, but HUFA production is generally inefficient and very limited (Gladyshev et al. 2009, 2011). HUFAs synthesised by microalgae in aquatic ecosystems are consumed by a variety of aquatic consumers, which in turn are consumed and ingested by terrestrial consumers, such as waterbirds and other terrestrial vertebrates (Ramos and González-Solís 2012, Karnovsky et al. 2012). Through these trophic connections, the important HUFAs are effectively transferred from aquatic to terrestrial habitats. Water birds in particular have been identified as key pathways of HUFA export from aquatic to terrestrial habitats, and global export rates of 19.3 to 167 kg HUFA km^{-1} $year^{-1}$ via birds have been estimated using preliminary data (e.g. Gladyshev et al. 2009, but see Connan et al. 2005, Wang et al. 2010, Richoux et al. 2010, Pollierer et al. 2010, Gladyshev et al. 2011, Torres-Ruiz et al. 2012, Karnovsky et al. 2012 for similar).

1.3 Stable isotopes as dietary tracers in birds

Over the past two decades, there has been an increase in the use of stable isotopes to determine the diets of waterbirds (e.g. Ramírez et al. 2011, Hahn et al. 2012, Karnovsky et al. 2012). Stable isotopes have been successfully used in avian-based studies ranging from temperate (e.g. Bearhop et al. 2000, Hipfner et al. 2010) to tropical (Awkerman et al. 2007, Cherel et al. 2008a) and even polar regions (Käkelä et al. 2006, 2007, 2010, Quillfeldt et al. 2010, Phillips et al. 2011, Wold et al. 2011). Several authors have provided over-views of the use of stable isotope methods in seabird investigations (Forero and Hobson 2003, Barrett et al. 2007, Bond and Jones 2009, Karnovsky et al. 2012) or the more general use of stable isotope techniques in avian or mammalian ecology (Inger and Bearhop 2008, Tollit et al. 2010, Hobson 2011). Initially used (Hobson and Wassenaar 1997) and further developed as a tool to study avian migrations (Kelly 2000, Michener and Kaufman 2007, Cherel et al. 2008b, Jaeger et al. 2010, González-Solís et al. 2011), this technique has resulted in enormous advances in the field of avian trophic ecology. Not only can the method provide information on basal diets, it can also inform us about foraging niches (Herrera et al. 2003; Fort et al. 2010; Ceia et al. 2012), trophic partitioning (Herrera et al. 2003), and seasonal shifts in the diets of birds (Karnovsky et al. 2008; Davies et al., 2009). The technique requires that different potential diet items are isotopically distinct, and that there is some information on the fractionation between food and consumer (Kelly 2000; Cherel et al. 2005). Unlike conventional stomach and faecal analyses or field observations, which provide incomplete diet information for brief periods, stable isotope ratios analysed from varying tissues can provide information on bird diet at very different time scales (Hobson and Clark 1992a, 1992b) Isotope ratios of feathers provide a record of a bird's diet during the growth period of the feather which will reflect the diet of the bird at the time of ingestion (Hobson and Clark 1992b, Cherel et al. 2000, Hobson 2011). In contrast, whole blood, red blood cells and blood plasma are continually being renewed through metabolic processes, and therefore will represent the diet of the bird over a relatively short time period (Hobson and Clark 1992b, Quillfeldt et al. 2008, Hobson 2011). When used inclusively, isotopic data from different tissues can provide an accurate composite picture of avian feeding habits. Karnovsky et al. (2008), Jaeger et al. (2010), and Cherel et al. (2013) demonstrated using stable isotope signatures from blood and feathers that the diet of seabirds varied significantly between summer and winter months.

While most stable isotope studies on waterbirds have focussed on the marine environment, there has been an increasing number of studies that have applied the technique to freshwater and terrestrial birds (Hobson and Wassenaar 1997, Herrera et al. 2003, Symes and Woodborne 2009, Wakelin et al. 2010, Brauns et al. 2011, Doucette et al. 2011, Kadye and Booth 2012, Hahn et al. 2012, Rodríguez and Herrera 2013). Herrera et al. (2003) used stable isotopes of nitrogen from whole blood samples to differentiate the trophic levels of tropical rainforest birds in Mexico, and was able to categorise 23 bird species based on their $\delta^{13}C$ signatures. This differentiation can be accomplished because C_3 and CAM/C_4 plants have different photosynthetic pathways that result in distinct $\delta^{13}C$ signatures within the same environment (C_3 mean $\delta^{13}C$=-27‰, CAM/C_4 mean $\delta^{13}C$=-12‰; Kelly 2000). The dietary analyses in this study gave some valuable insights into niche partitioning and habitat compartmentalisation by birds, in an ecosystem that is well-known for its high inter-specific competition (Herrera et al. 2003).

1.4 Trophic niche ecology

The concept of the ecological niche dates back to the middle of the twentieth century, and has captivated the attention of ecologists ever since. First described as an "*n*-dimensional hypervolume" by Hutchinson (1957), the niche concept is important in ecology for understanding species interactions and the structuring of communities (Syväranta et al. 2013). Originally, the ecological niche was described as the ecological space expressed by all resources exploited by a population or species (Hutchinson 1957), but it was later realised that such a volume would be impossible to quantify (Semmens *et al.* 2009). Ecologists have realised that the feeding/trophic niche (Elton 1927) is potentially more feasible to measure, whereby the dietary diversity of a consumer is measured and compared to others. In this approach, a set of *n* variables that represent biologically important and independent axes are identified, and the hypervolume is defined by a set of points within this *n*-dimensional space that reflects suitable values of the variables (e.g. temperature or food size; Blonder et al. 2014). The trophic niche hypervolume concept is widely used in comparative biology (e.g. Pigliucci 2007) and evolutionary biology (e.g. Gavrilets 2004; Litsios et al. 2012; Jackson & Britton 2013). Traditionally, gut content analysis has been used as a measure of trophic niche width, but this invariably requires laborious examinations of the diets of many individuals in a population over an extended period of time to account for temporal fluctuations in diet (Semmens et al. 2009, Blonder et al. 2014). Stable isotope ratios represent a more economical and integrative approach to trophic niche quantification.

The isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), for example, are widely used by ecologists to determine niche space of species, because they provide time-integrated information of assimilated diet over varying time lengths (depending on tissue turnover), and information on sources of carbon and trophic level. In addition, the $\delta^{13}C$ and $\delta^{15}N$ values can often provide information on habitat utilisation, as different habitats may contain different prey items (Giraldeau and Caraco 2000).

1.5 Fatty acids as dietary tracers in birds

Conventional diet techniques, as previously mentioned, are plagued by inherent biases and downfalls (Williams and Buck 2010). Consequently, scientists investigating avian ecology are relying ever increasingly on molecular trophic markers, such as DNA analysis (e.g. Deagle & Tollit 2007, Yoccoz 2012), stable isotope analysis (e.g. Hobson 1990; Christie et al. 2008; Wold et al. 2011; Doucette et al. 2011) and fatty acid profiles (e.g. Iverson et al. 2004, 2007, Käkelä et al. 2006, 2007, 2010, Maranto et al. 2011, Karnovsky et al. 2012). Fatty acid profiles have been used successfully to describe temporal and spatial diet variability (e.g. Karnovsky et al. 2008; Wang et al. 2009; Käkelä et al. 2010), specialised feeding regimes (e.g. Steinmetz et al. 2003; (Budge et al. 2007) and intra- (e.g. Williams et al. 2008, Richoux et al. 2010) and inter-specific niche segregation (e.g. Dahl et al. 2003). The primary focus of research utilising fatty acid profiles to describe the diet of consumers has been based in the marine environment, predominantly because polyunsaturated fatty acids (PUFAs) are derived from phytoplankton and are subsequently transferred through the food web (Williams and Buck 2010). Because of modification, selective catabolism/storage and de novo synthesis of fatty acids, fatty acid analysis of consumers will never directly match the composition of prey items (Williams and Buck 2010). However, the strong influence of diet on the fatty acid composition of various tissues in consumers permits qualitative (and sometimes quantitative) dietary inferences to be made (reviewed by Budge et al. 2006). The principal assumption underpinning the use of fatty acids in trophic ecology is that the effects of consumer metabolism on fatty acid profiles are at least partially predictable (Williams and Buck 2010). Several studies have questioned this underlying assumption, contradicting the notion that fatty acid signatures can be suitably used as diet tracers, because fatty acid metabolism is too variable to be predictable (Grahl-Nielson et al. 2003). Despite the divergent views on the use of fatty acids as reliable indicators of diet in all consumers, there is a plethora of literature that supports the effectiveness of fatty acids as a dietary tracer tool (Thiemann et al. 2009).

Fatty acids can be used effectively to determine the diets of consumers (Käkelä et al. 2009, 2010, Kohler et al. 2011), but certainly the technique becomes less reliable with increases in trophic level. As such, numerous researchers have recognised that additional techniques such as stable isotope analysis should be used in conjunction with fatty acid profile analysis (e.g. Barrett et al. 2007; Connan et al. 2007; Iverson et al. 2007; Thiemann et al. 2008; Williams et al. 2008; Karnovsky et al. 2008; Käkelä et al. 2010).

1.6 Thesis overview

South African estuaries are biologically rich and diverse in species, with many species being geographically endemic (Turpie et al. 2002). South African estuaries are well known for their high abundance and richness of bird species (Hockey et al. 1992, Hockey and Turpie 1999). Additionally, South African estuaries play a role as over-wintering sites for numerous European and Asian bird species (Turpie et al. 2002). Consequently, estuaries play a pivotal role as a primary foraging ground for both local and migratory species (Moreira 1997). These aquatic-feeding birds (henceforth referred to as waterbirds), fill a myriad of feeding guilds and can have a significant predatory impact on invertebrate and nekton prey communities (Blaber 1973, Moreira 1997, Kaletjta-Summers et al. 2009, Bergamino et al. 2012), and therefore play a fundamental role in mass and energy fluxes (Moreira 1997). Cross-habitat subsidies, whereby consumers that originate in one habitat ingest food sources that originate in another habitat, is a relatively new concept in ecology, but scientists are increasingly recognising the importance of the cross-habitat subsidies to ecosystem energy fluxes and food web dynamics (Matthews and Mazumder 2012, Polis et al. 1997). To better understand how cross-habitat subsidies between terrestrial and aquatic habitats may influence reciprocating food webs, it is required to first accurately determine the diets of predators to assess how and where predatory pressure is exerted. Stable isotopes and lipid profiles have been used extensively to determine the diet of avian consumers (see above for examples). However, the combination of these tools has never been used to determine the diets of waterbird consumers in estuarine ecosystems in South Africa. Furthermore, there are few studies that have investigated the temporal stochasticity of the diet of waterbirds, and how or why the diet of a single species may display seasonal variation.

This thesis primarily aims to determine the role that waterbirds play as consumers in aquatic habitats and determine the seasonal changes in the diets of several common waterbird species in the Kowie Estuary, South Africa.

Chapter 2 investigates the population dynamics, energy consumption and fresh matter consumption of waterbirds in the Kowie Estuary to establish a baseline measure of their predatory effect on the aquatic food web.

In Chapter 3, the diet of several waterbird species were determined using $\delta^{15}N$ and $\delta^{13}C$ stable isotopes of blood tissue sampled from individual birds over 12 months. Using abundance data of potential prey items, Chapter 3 includes an investigation of how prey abundance may influence diet choice and potential diet shifts in waterbirds. Birds offer a unique opportunity to study their diet over extended periods of time, particularly when using stable isotopes analysis. Inert body tissues such as feathers and claws offer stable isotope values that represent diet at the time of the deposition of the tissue. In some instances, such as primary flight feathers, stable isotope signatures may reflect diet as far back as 12 months from time of collection. Consequently, Chapter 4 represents an investigation of several body tissues with dissimilar turn-over rates to explore the diet of individuals over an extended period of time. These tissues provide insight into the diet of individuals over a period of 3 to 12 months from time of collection. Furthermore, they provide insight into how sampling different body tissues might influence the determination of waterbird diet.

Chapter 5 represents a study of seasonal variability in the lipid profiles of soft body tissues and determines whether lipid profiles can be effectively used to ascertain the seasonal changes in the diets of waterbirds and how lipids are transferred through the trophic levels from basal resources to waterbirds in the upper trophic levels. An overview of general dietary trends and isotopic patterns, and an evaluation of waterbirds as predators in aquatic food webs are presented in Chapter 6, along with a critique of methods used and plausible alternative methods for future studies. Explanations and plausible theories are presented in an attempt to unravel the mechanisms that may drive diet shift in waterbird populations feeding in estuarine ecosystems.

1.7 Ethics statement

All birds were collected and euthanased with permission from the Rhodes University ethical committee (ZOOL-09-2012), and the South African Department of Environmental Affairs (CRO 52/13CR and CRO 53/13CR). All birds were euthanased instantly and painlessly, and collected and handled by experienced persons only.

Population dynamics, feeding rates and energy consumption of selected waterbirds within the lower reaches of the Kowie Estuary

2.1 Introduction

Determining the influence of consumers on prey populations in aquatic food webs is fundamentally necessary to our understanding ecosystem dynamics (Moreira 1997, Wootton 1997, Steinmetz et al. 2003). Conventional models of aquatic food webs assume that fish occupy the top trophic level and are the most important biotic determinant of trophic abundance lower down in the food web (e.g. Fry 1991, Wellborn et al. 1996). However, many terrestrial consumers, including many bird species, feed in aquatic systems, and therefore are components of trophic levels in aquatic food webs (e.g. Moreira 1997, Steinmetz et al. 2003, Rosa et al. 2008, Bergamino et al. 2012). They thus have the potential to be important drivers of aquatic food web dynamics. Estuaries are often bear the brunt of anthropogenic activities, such as habitat destruction, pollution and over exploitation (e.g. Courrat et al. 2009, Deegan et al. 1997, Milner et al. 2007, Selleslagh et al. 2012, Whitfield et al. 2012). Studying predator-prey dynamics in these ecosystems will improve our comprehension of predator-prey community structure (Holt 1977) and how these communities fluctuate over space and time. More particularly, there is a need to ascertain the effects of all predatory pressures exerted on the aquatic food webs in estuaries. South African estuaries support a high diversity of aquatic-feeding birds, with approximately 162 species of birds recorded from 13 orders (Hockey and Turpie 1999, Turpie et al. 2002). The great diversity of birds in South African estuaries can be attributed to the wide variety of habitats that estuaries provide, which include mudflats, shallow banks, reed beds, deep water channels and salt marshes (Hockey and Turpie 1999). These habitats, in turn, provide a rich diversity of food sources. Large numbers of shorebirds depend on intertidal areas of estuaries for feeding during the non-breeding season, and these areas often support over-wintering bird populations (Granadeiro et al. 2007). South African estuaries support at least 345 000 non-passerine individuals during the summer months, of which approximately 225 000 belong to the order Charadriiformes (Martin and Baird 1987, Hockey and Turpie 1999, Froneman et al. 2011). Because waterbirds congregate in very high abundances in South African estuaries, the effect that they have on prey communities is undoubtedly significant. When waterbirds occur in high abundances, such as in estuaries during the breeding season or when birds are preparing for/returning from migration, the predatory pressure from waterbirds can significantly deplete prey community abundances (Rosa et al. 2008).

Kalejta-Summers et al. (2009) described how waterbirds feeding in the Rietvlei lagoon accounted for up to 52% of the total benthic invertebrate removal. Consequently, there is a growing body of literature that highlights how waterbirds remove a substantial proportions of benthic macrofauna (e.g. Szekely and Bamberger 1992, Moreira 1997, Rosa et al. 2008) and directly alter the abundance of fish populations (e.g. Blaber 1973, Crowder et al. 1997, Steinmetz et al. 2003, Bergamino et al. 2012). Typically, determining the impact of waterbirds of prey communities and the role of waterbirds in ecosystem energy fluxes has been achieved through exclusion experiments (e.g. Boates and Smith 1979, Goss-Custard 1980, Quammen 1984, Marsh 1986, Raffaelli and Milne 1987, Szekely and Bamberger 1992). Through such experiments, waterbirds have been implicated as important drivers of energy and mass fluctuations in aquatic food webs (Moreira 1997, Ramos and González-Solís 2012). Alternatively, the effect of consumers on prey populations can be estimated through daily consumption rates coupled with population counts (e.g. Goss-Custard et al. 1991, Szekely and Bamberger 1992, Kalejta-Summers et al. 2009, Froneman et al. 2011). The use of dietary estimations is a relatively simple method compared to exclusion experiments, and it is non-intrusive in the estuarine environment. Coupled with population counts, energy consumption and daily feeding rate estimations offer valuable insights into the roles that avian consumers play in aquatic food webs, and they allow for the evaluation of seasonal effects on those food webs.

I posed the following questions to ascertain the role and degree of predatory pressure that waterbirds exert on the Kowie Estuary food web; 1) what is the temporal variation in the total abundance of waterbirds in the Kowie Estuary, 2) what is the community composition of waterbirds in the Kowie Estuary, and 3) what is the temporal variation in the total energy consumption of waterbirds in the Kowie Estuary? This baseline information is used in conjunction with trophic tracer data in later sections (Chapters 3 and 4).

2.2 Methods

2.2.1 Study Site and data collection

The Kowie Estuary is a permanently open system that is partially located in the town of Port Alfred (33° 36’ 11”S; 26° 54’10”E), on the South-East coast of South Africa. It is one of the longest tidal estuaries in South Africa, where marine water can extend up to 21 km from the estuary mouth (Heinecken and Grindley 1982). The climate in the region is classified as warm temperate, with annual precipitation occurring primarily in the summer months (Heinecken and Grindley 1982, Whitfield 2000).

The most abundant bird species were counted at three locations (**Fig 1**). Anecdotal evidence coupled with observations made during preliminary field trips suggested that these sites were most commonly frequented by waterbirds and they were best suited for field observations. Topographically, the Kowie River is bounded by high, steep cliffs along much of its length to the lower reaches of the estuary. Thus there are few other suitable mudflat feeding sites available to waterbirds. All three sample sites are natural alluvial mudflats subjected to the ebb and flow of marine water entering the estuary with daily tides. Population counts were conducted from June 2013 until May 2014, ten times a month at the three locales described. To avoid double counting, the highest number of observed individuals per species recorded on the sample day was taken as the total count.

Fig 1: Sample sites (dotted areas) along the lower reaches of the Kowie Estuary, Port Alfred. 1 = primary tidal mudflat (61.06 ha; 33°35’05 S 26°52’35 E); 2 = tidal mudflat 2 (1.34 ha, 33°35’40 S 26°53’10 E); 3 = tidal mudflat 3 (3.28 ha; 33°53’33 S 26°53’26 E).

2.2.2 Data analysis

Analyses of variance (ANOVA) with Tukey *post-hoc* tests were used to determine any effects of time on population size, and the total monthly energy consumption (TEC) and the fresh matter intake (FMI) for each species. Field metabolic rate (FMR) was used as a proxy for daily energy consumption requirements (kilojoules per day) (Nagy 1987), while FMI was the calculated minimum mass of diet items that needed to be consumed to obtain the daily energy requirements (Nagy 2005). Monthly energy consumption was calculated for each population using the equation, $FMR = 10.9\ BM^{0.640}$, where BM = body mass in grams (Nagy 1987). **Table 1** provides information on mean body mass, feeding guild and daily energy requirements of individuals from resident waterbird species observed along the Kowie Estuary. This FMR value was multiplied by the mean monthly population count for each species, to obtain mean daily TEC per species.

The mean daily TEC value from all populations were pooled to obtain total community mean TEC per day. Monthly FMI was calculated using the equation, $FMI = (0.648\ BM^{0.651}) \times 3.448$, where BM = body mass in grams, and 3.448 is a universal standard unit to convert dry mass to wet mass of diet items, presuming that diet organisms comprise 70% water (Nagy 1987). FMI data were pooled as per TEC to obtain a monthly mean FMI per day. Diversity of waterbirds was calculated during each season using the Shannon-Weiner diversity index. All statistical analyses were performed in R (v 3.03 for Windows) and all graphics were completed in SigmaPlot™ (v10.0 for Windows).

Table 1: Classification of diet of resident waterbird species in the Kowie Estuary (Hockey et al. 2005)

Family	Common name	Species name	Mean mass (g)	Feeding guild	Required energy consumption (kJ/day)
Anhingidae	Reed Cormorant	*Microcarbo africanus*	559	Fish	585
Ardeidae	Little Egret	*Egretta garzetta*	346	Fish	422
	Black Headed Heron	*Ardea melanocephala*	1449	Mixed	1119
	Grey Heron	*Ardea cinerea*	1449	Mixed	1119
	Cattle Egret	*Bubulcus ibis*	370	Mixed	360
Threskiornithidae	African Spoonbill	*Platalea alba*	1600	Mixed	1197
Anatidae	Cape Shoveller	*Anas smithii*	603	Invertebrates	616
	Cape Teal	*Anas capensis*	♂419, ♀380	Invertebrates	344
	Egyptian Goose	*Alopochen aegyptiacus*	1872	Omnivore	1556
	Yellow-Billed Duck	*Anas undulata*	823	Omnivore	849
Recurvirostridae	Black winged Stilt	*Himantopus himantopus*	170	Invertebrates	260
Charadriidae	Ruff	*Phylomachus pugnax*	♂170, ♀100	Invertebrates	172
	Common Ringed Plover	*Charadrius hiaticula*	55-72	Invertebrates	115
	Blacksmith Lapwing	*Vanellus armatus*	163	Invertebrates	253
	Curlew Sandpiper	*Calidris ferruginea*	57-79	Invertebrates	131
	Greenshank	*Tringa nebularia*	170-230	Invertebrates	276
	Common Sandpiper	*Actitis hypoleucos*	50-70	Invertebrates	115

2.3 Results

2.3.1 Census counts

Population counts revealed that the Kowie Estuary was dominated by 17 bird species that encompassed four orders (Charadriiformes, Aseriformes, Ciconiiformes and Suliformes), and five families (Anatidae-ducks, Ardeidae-herons and egrets, Recurvirostridae-avocets and stilts, Charadriidae-shore birds and Anhingidae-darters). The total count of all observed birds was highest in December 2013, with a mean total of 513 birds observed. Conversely, the lowest total counts of birds occurred in July and August 2013 (133 individuals). The mean number of birds observed were significantly higher from October 2013 to February 2014 than all other months of the study year (June-October2013 and March-May 2014; $F_{11} = 55$, $p < 0.001$, **Fig 2**). Total counts of all birds in October 2013 was also lower than those in November 2013 ($p = 0.079$), December 2013 ($p<0.001$), January 2014 ($p < 0.001$) and February 2014 ($p < 0.001$).

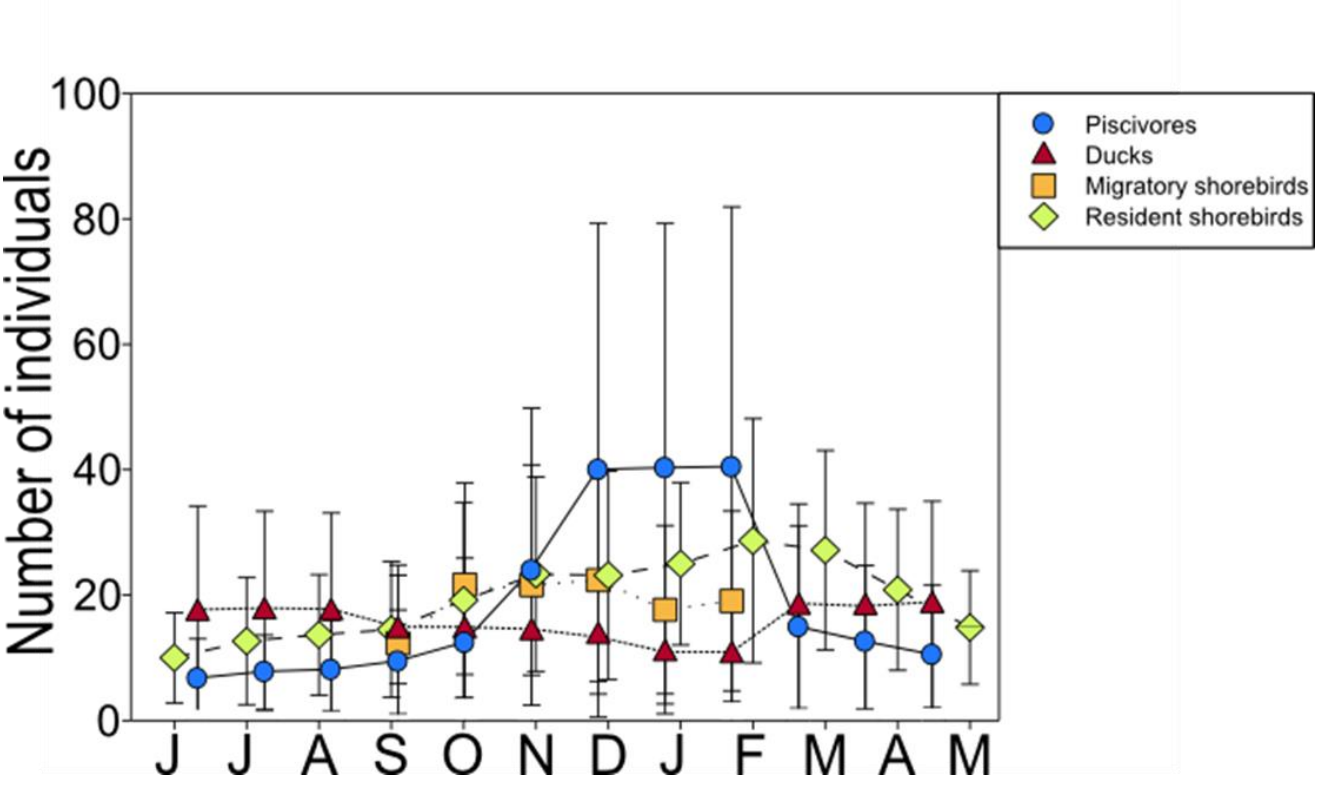

Fig 2: Mean (±SD) monthly total census counts calculated for piscivores (Pelicaniformes; blue circles), ducks (Anseriformes; red triangles), migratory shorebirds (Charadriiformes; gold squares) and resident shorebirds (Charadriiformes; green triangles).

Similarly, fewer birds were observed in November 2013 compared with December 2013 ($p = 0.004$) and February 2014 only ($p < 0.006$). There was no significant difference in total bird counts between November 2013 and January 2014 ($p = 0.378$). The mean monthly totals of invertebrate feeding and piscivorous birds displayed a similar trend as those of the total community counts. Invertebrate feeders peaked in abundance in November 2013 (228 individuals) (**Fig 2**), while piscivores peaked in abundance in December 2013 (289 individuals) at the height of their breeding season. ANOVA showed that for invertebrate feeders, abundances during October 2013 to February 2014 were significantly higher compared with all other months of the study year ($F_{11} = 17$, $p<0.001$). Similarly, piscivore abundance was greatest from November 2013 to February 2014 ($F_{11} = 37$, $p < 0.001$). The increase in the abundance of piscivorous birds in the estuary was rapid in the spring and summer months (less than 50 to a maximum of 289 individuals in December), while the abundance of invertebrate feeders decreased over the same time period (228 individuals in November 2013 to 202 individuals in January 2014).

2.3.2 Daily energy intake (DEI) and fresh matter intake (FMI)

Energy consumption based on total counts peaked in December 2013 (mean ± SD = 7.44×10^6 kJ/day ± 2.71×10^6 kJ/day), with piscivorous birds contributing 54% to the total energy consumption (**Fig 3**). The lowest energy consumption calculated occurred in June 2013 (mean ± SD = 2.17×10^6 kJ/day ± 1.33 $\times 10^6$ kJ/day), with invertebrate feeding birds contributing 69 % of the total energy consumption. Similarly, invertebrate feeding birds contributed the most to the overall energy consumption during September 2013 (73%), while piscivores contributed the most to total energy consumption during January 2014 (55%). The energy consumption by all waterbirds was significantly higher from December to February compared to all other months of the study ($F_{11} = 7$, $p < 0.001$). The calculated fresh matter (FMI) intake based on the total counts of all observed species displayed a similar trend to energy consumption, with FMI being greatest in December 2013 (mean ± SD = 5.5×10^4 g/day ± 3.4×10^3 g/day), closely followed by February 2014 (mean ± SD = 4. 9 x 10^5 g/day ± 3310 g/day). ANOVA revealed that, as with energy consumption, FMI in December 2013 to February 2014 was higher than all other months of the study year ($F_{11} = 8$, $p < 0.001$, **Fig 3**).

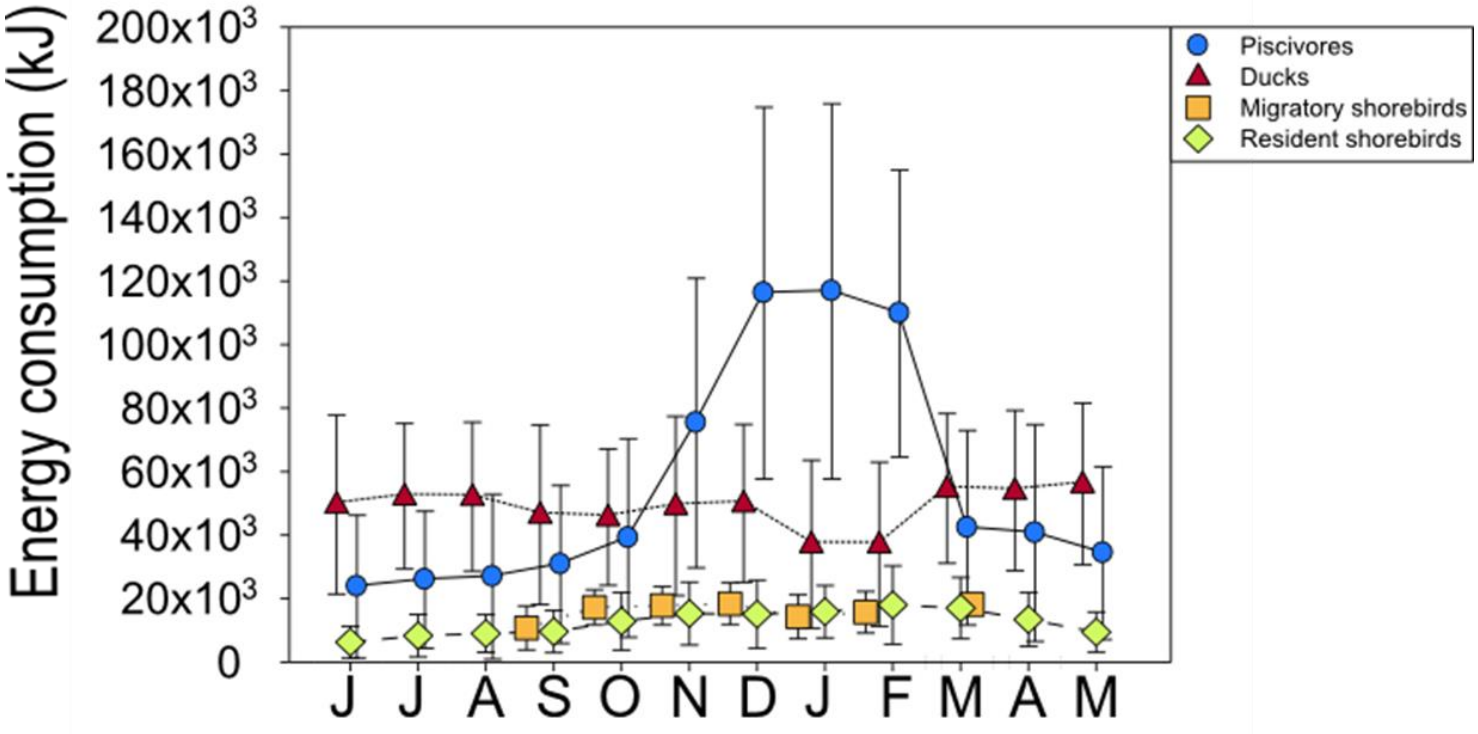

Fig 3: Mean (±SD) total energy consumption/day calculated for piscivores (Pelicaniformes; blue circles), ducks (Anseriformes; red triangles), migratory shorebirds (Charadriiformes; gold squares) and resident shorebirds (Charadriiformes; green triangles).

Table 2: Population counts along the lower reaches of the Kowie Estuary (June 2013 – May 2014)

Common name	Species name		v	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Little Egret	*Egretta garzetta*	mean	4	9	10	16	15	30	100	102	105	6	5	7
		min	0	5	4	10	9	20	90	94	90	3	2	1
		max	12	14	15	20	21	43	110	112	116	9	11	12
Reed Cormorant	*Microcarbo africanus*	mean	7	9	10	6	9	7	26	26	28	25	17	8
		min	0	4	0	1	2	2	4	6	5	2	1	0
		max	17	16	18	21	15	18	44	53	51	49	34	17
Black Headed Heron	*Ardea melanocephala*	mean	3	5	6	6	7	9	12	16	14	7	9	6
		min	0	0	0	0	0	0	0	0	4	0	1	0
		max	8	9	15	10	17	24	30	33	36	16	14	13
Grey Heron	*Ardea cinerea*	mean	5	5	4	5	5	15	19	13	4	6	5	4
		min	1	0	0	0	0	3	2	4	0	0	0	0
		max	10	10	9	10	10	26	38	35	9	12	17	9
Cattle Egret	*Bubulcus ibis*	mean	12	14	13	17	31	70	75	66	82	38	30	30
		min	3	4	2	4	13	54	53	50	65	30	21	20
		max	23	22	30	29	56	93	104	100	100	48	40	42
African Spoonbill	*Platalea alba*	mean	5	4	2	4	4	5	3	3	6	5	3	6
		min	0	0	0	0	0	0	0	0	0	0	0	0
		max	9	8	6	9	8	10	8	10	11	11	15	12
Cape Shoveller	*Anas smithii*	mean	41	42	41	21	20	13	7	3	4	44	43	43
		min	30	32	33	12	12	5	3	0	0	36	36	36
		max	55	50	49	32	28	20	12	7	8	49	50	51
Cape Teal	*Anas capensis*	mean	12	11	11	12	13	13	13	14	12	13	10	12
		min	6	6	4	5	4	6	5	4	4	6	4	7
		max	19	19	19	20	20	20	20	20	20	18	20	17
Ruff	*Phylomachus pugnax*	mean	0	0	0	27	48	54	52	35	38	0	0	0
		min	0	0	0	5	40	44	41	25	26	0	0	0
		max	0	0	0	40	56	63	60	50	52	0	0	0

Common Ringed Plover	*Charadrius hiaticula*	mean	0	0	0	14	21	17	21	16	19	0	0	0
		min	0	0	0	4	8	10	11	5	6	0	0	0
		max	0	0	0	21	33	29	36	25	28	0	0	0
Blacksmith Lapwing	*Vanellus armatus*	mean	10	17	19	23	30	35	31	28	34	26	28	16
		min	3	4	8	5	7	10	11	16	12	17	9	4
		max	18	27	26	30	44	43	43	36	40	37	35	29
Curlew Sandpiper	*Calidris ferruginea*	mean	0	0	0	6	8	9	8	10	8	0	0	0
		min	0	0	0	0	0	0	1	4	0	0	0	0
		max	0	0	0	12	16	20	19	17	21	0	0	0
Greenshank	*Tringa nebularia*	mean	0	0	0	0	6	5	7	7	7	0	0	0
		min	0	0	0	0	0	0	0	0	1	0	0	0
		max	0	0	0	0	12	11	14	13	14	0	0	0
Black winged Stilt	*Himantopus himantopus*	mean	10	9	9	10	11	18	15	21	30	27	15	13
		min	1	0	0	2	3	3	1	7	1	5	5	7
		max	18	19	20	17	20	31	38	42	55	51	33	20
Common Sandpiper	*Actitis hypoleucos*	mean	0	0	0	19	21	21	23	18	22	0	0	0
		min	0	0	0	8	14	11	12	10	14	0	0	0
		max	0	0	0	29	28	30	31	30	30	0	0	0
Egyptian Goose	*Alopochen aegyptiacus*	mean	12	12	11	10	11	14	16	12	14	13	14	15
		min	0	5	5	6	5	6	8	0	2	4	3	3
		max	18	19	20	20	15	25	27	20	20	21	22	22
Yellow-Billed Duck	*Anas undulata*	mean	4	4	5	12	16	15	17	16	15	5	4	6
		min	0	0	0	2	8	9	12	10	6	0	0	0
		max	10	10	10	22	22	24	23	24	21	10	8	10

2.3.3 Diversity indices and comparison of ecosystems

The diversity index calculated for the Kowie Estuary was higher than those calculated for other South African ecosystems that have used similar energy consumption methods (Table 3). Similarly, the Kowie Estuary had a higher diversity index than the Dutch portion of the Wadden Sea (H' = 2.37) and the Tagus Estuary in Portugal (H' = 1.37), but was lower than the diversity of waterbirds in the Tees Estuary (H' = 2.84) and the Ythan Estuary in the United Kingdom (H' = 3.13, **Table 3**). There was no correlation between waterbird diversity and energy consumption amongst South African estuaries ($p = 0.238$), or amongst European estuaries ($p = 0.226$), nor amongst South African and European estuarine ecosystems ($p = 0.133$).

Table 3: Examples of calculated energy consumption by waterbirds in estuaries

Location	Calculated energy consumption	Shannon-Weiner diversity	Reference
Kowie Estuary, South Africa	127 kJ m^{-2} $year^{-1}$	2.62	This study
Riet River, South Africa	366 kJ m^{-2} $year^{-1}$	1.73	Froneman et al. 2011
Rietvlei, South Africa	270 kJ m^{-2} $year^{-1}$	2.46	Kalejta-Summers et al. 2009
Swartkops Estuary, South Africa	355 kJ m^{-2} $year^{-1}$	2.33	(Martin and Baird 1987)
Langebaan Lagoon, South Africa	142 kJ m^{-2} $year^{-1}$	2.27	Wolff and Smit 1990
Wadden Sea, Holland	104 kJ m^{-2} $year^{-1}$	2.37	Evans et al. 1984
Tees Estuary, United Kingdom	367 kJ m^{-2} $year^{-1}$	2.84	Evans et al. 1984
Ythan Estuary, United Kingdom	874 kJ m^{-2} $year^{-1}$	3.13	Baird et al. 1985
Tagus Estuary, Portugal	103 kJ m^{-2} $year^{-1}$	1.37	Moreira 1997

2.4 Discussion

The Kowie Estuary contained a relatively low abundance (> 450 individuals) but a high diversity (H' = 2.62) of waterbird species, which is consistent with other previous studies conducted in both intermittently open estuaries and permanently open estuaries in South Africa (e.g. Jackson 1984, Boshoff et al. 1991, Hockey and Turpie 1999, Granadeiro et al. 2006, Froneman et al. 2011, Hockey et al. 2012, Terörde and Turpie 2012).

There was higher abundance and diversity of waterbirds throughout the warm spring and hot summer months (which included the presence of non-breeding migratory species Ruff, Common sandpiper, Curlew Sandpiper and Greenshank) compared to the autumn and summer months along the Kowie Estuary. These migrants arrived in South Africa in large numbers (22% - 36% of total counts for all waterbird species) and were responsible for a high proportion of the calculated energy consumption during spring and summer (**Fig 3**). The limited number of large mudflats in the Kowie Estuary may have contributed to the relatively low waterbird numbers compared to other South African estuaries (e.g. Martin and Baird 1987, Wolff and Smit 1990, Kalejta-Summers et al. 2009), consequently leading to a lower total energy consumption compared to other South African estuaries (see **Table 3**).

Few studies have calculated the energy consumption of fish predators on prey populations, so no comparisons can be drawn between consumer types. However, Crowder et al. (1997) found that piscivorous birds had a lower predatory effect on baitfish species than a fish predator, while Rosa et al. (2008) found that waterbirds and fish predators had equally strong deleterious effects on an aquatic invertebrate community in the Tagus Estuary, UK. The increasing abundances of piscivorous species, such as Grey Heron, Black-headed Heron, Little Egret and Cattle Egret during the summer months (late November 2013 to February 2014) were associated with their breeding cycles and use of large nesting sites in the Kowie Estuary (personal observations 2013, 2014). Increased nekton productivity during the summer months may also be contributing factor to the increased abundance of piscivorous birds in the estuary (Whitfield et al. 1994). However, the high energy consumption and presumably high predatory pressure exerted by the piscivorous species on nekton was short-lived (November to February), while invertebrate feeding waterbirds was the most abundant feeding guild in the Kowie Estuary for nine months of the year. The effects that aquatic feeding consumers can have on prey communities can be indirect, whereby the effect of consumers is mediated through more than one trophic link and possibly lead to trophic cascades (Jones et al. 1994, Wooton 1994, Bruno and Bertness 2001). Strong indirect effects of consumers often propagate downward and laterally along links in the food web, causing substantial changes in abundance of organisms elsewhere in the food web (Menge and Lubchenco 1981, Sih et al. 1985). The focus of studies investigating the top-down pressure of consumers on prey populations in aquatic habitats has been skewed towards fish (Steinmetz et al. 2003). Yet, terrestrial-based consumers such as birds and otters play functionally significant roles in regulating prey populations in aquatic food webs (Moreira 1997, Steinmetz et al. 2003). Additionally, waterbirds and seabirds alike perform several crucial roles in aquatic ecosystems, from regulators of prey species abundances to vectors of nutrient transfer (Diamond and Devlin 2003, Piatt et al. 2007, Sydeman et al. 2014).

Studies that outline and evaluate the simultaneous consequences of seabird and waterbird trophic interactions on aquatic ecosystem food webs are relatively uncommon, however (Bergamino et al. 2012). Moreover, the impact of these consumers on fisheries is a controversial issue of long-standing in the field of conservation and fisheries management (Doucette et al. 2011). This study has not been able to quantify the full effects that avian consumers have on prey community abundance and distribution, but the calculated energy consumption of waterbirds infers that the effect of waterbirds on prey communities can be expected to be significant. Anthropogenic activities and disturbance in estuaries often leads to a significant decrease in waterbird abundance (Turpie 1995, Hockey and Turpie 1999, Turpie et al. 2002). It must be noted that the values presented are estimates of consumption rates and energy intake, and assume that all birds observed were feeding within the estuarine environment. However, some birds may have obtained food from the terrestrial environment or adjacent estuarine ecosystems. Additionally, presuming that all individuals are actively feeding in the Kowie Estuary alone may lead to some significant over-estimations of calculated energy consumption. However, it is extremely difficult to accurately observe and assess the degree of active feeding of all waterbird species individuals on a large scale. The values presented here do give some insight into the predatory pressure that waterbirds may exert on aquatic communities and structure in a South African estuary.

Waterbirds play a pivotal role in the energy flux dynamics of ecosystems while also exerting significant predatory pressure that shapes and regulates prey communities (Drossel et al. 2001). Understandably, the magnitude of the predatory pressure that waterbirds impose on aquatic prey communities is difficult to accurately measure. Although energy consumption calculations are simplistic and broad-based, future studies could possibly incorporate similar energy consumption calculations to provide some measure of the predatory effect that waterbirds have on aquatic prey communities. Furthermore, quantification of prey biomass in conjunction with exclusion experiments would further provide some measure of the predatory pressure that waterbirds have on prey communities. Direct comparisons between the predatory effect of fish and waterbirds on prey communities is lacking (but see Rosa et al. 2008), and is an area of research that requires attention. Estimates of energy consumption of waterbirds and/or energy flow from prey to predators contribute significantly to our understanding of predator-prey dynamics and ecosystem functioning, but may not truly reflect the functional implications of trophic links between waterbirds and their aquatic prey. Accurately determining the diet of waterbirds therefore becomes a crucial link to better understanding the full predatory impact of waterbirds on aquatic food webs.

Seasonal diet shifts in waterbirds revealed by stable isotope analysis

3.1 Introduction

Understanding the dynamics of predator–prey relationships in space and time is important to improve our knowledge of community structure and evolution (Paine 1980). The accurate determination of animal diets is imperative if we are to understand how consumers influence prey community structure and abundance (Tilley et al. 2013). More specifically, how consumers utilise dietary resources and interact with each other when foraging within a given environment is important for unravelling the pressures that consumers exert on specific prey communities. Seabirds and waterbirds have a significant effect on prey communities in aquatic habitats (e.g. Rosa et al. 2008). Seabirds have been the primary focus of research on avian diets, especially in regions where seabirds congregate in high densities to breed (Barrett et al. 2007). Waterbirds often congregate in high abundances (> 1 million individuals in some instances) in estuarine ecosystems around the world (see Smit 1981, Hockey et al. 1992). However, investigations into the variation in diet and trophic interactions between waterbird species on intertidal mudflats is lacking, despite literature emphasising the significant influence that waterbirds exert on invertebrate (Szekely and Bamberger 1992, Mendonça et al. 2007, Rosa et al. 2008) and nekton (e.g. Blaber 1973, Steinmetz et al. 2003, Bergamino et al. 2012) communities in aquatic food webs (Moreira 1997, but see Chapter 2 for examples and references).

Stable isotopes have been successfully used to infer diets of predators, such as Bluefin tuna, sharks, wolves, seabirds and waterbirds (e.g. Barrett et al. 2007, Karnovsky et al. 2008, Martínez del Rio et al. 2009, Collier and Lyon 2010, Cherry et al. 2011, Polito et al. 2011, Ramírez et al. 2011, Phillips 2012). Stable isotope techniques have allowed ecologists to increase the level of accuracy of detail when studying the function of complex food webs than previously achieved through observational data alone (Boecklen et al. 2011, Karnovsky et al. 2012). Applications of stable isotope ratios (typically of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in food web ecology take advantage of natural variation in stable isotope ratios, and the underlying aspects of a species trophic niche, which the variation reflects (Layman et al. 2007). Groups of food items available to organisms often differ in their isotopic signatures, and one can visualize the resource space as an area with isotopic values as coordinates (Newsome et al. 2007).

Stable isotope analysis has been successfully used to assign species to trophic positions in food webs (e.g. Hobson et al. 1994, Vander Zanden and Rasmussen 1999, Post 2002, Herrera et al. 2003, Symes and Woodborne 2009, Semmens et al. 2009, Ingram et al. 2009, Anderson and Polis 2012, Carlisle et al. 2012, Layman and Allgeier 2012, Rodríguez and Herrera 2013), to elucidate patterns of resource acquisition and allocation (e.g. Cherel et al. 2005, O'Brien et al. 2005, Waas et al. 2010), and to characterize niche properties (Cherel et al. 2007, Newsome et al. 2007, Quevedo et al. 2009, Williams and Purves 2011, Jackson et al. 2011, 2012, Doucette et al. 2011, Cummings et al. 2012, Litsios et al. 2012, Fink et al. 2012, Jackson and Britton 2013). Stable isotope techniques have provided ecologists with an avenue to quantitatively explore the trophic niche of animals (Syväranta et al. 2013). First defined by Hutchinson (1957) as the "*n*-dimensional hypervolume of resources available to organisms", the ecological niche concept has been fundamental for ecologists interested in studying trophic relationships of consumers and prey (Bearhop et al. 2004, Newsome et al. 2007, Semmens et al. 2009). Analyses of ecological/trophic segregation seek to explain how species differ in their use of limited resources (Navarro et al. 2013) whereby, according to the principle of competitive exclusion, ecologically-similar species are expected to partition their use of resources in a defined environment, leading to niche divergence (Gause 1973, Navarro et al. 2013). Consequently, the determination of temporal variation in the size, shape and distribution of a species' trophic niche can reveal the stochastic nature of resource use, and evidence of intra- and interspecific competition amongst consumers (Bearhop et al. 2004, Martínez del Rio et al. 2009a, Araújo et al. 2011, Gavrilchuk et al. 2014).

Chapter 2 estimated, from population data, the energy consumption of waterbirds in the Kowie Estuary relative to other South African and European estuaries. Energy calculations alone do not provide much information on the pressures that waterbirds exert on invertebrate and nekton communities. As such, accurate determinations of consumer diet and prey availability are required to elucidate how waterbirds influence prey communities in estuarine habitats. Using $\delta^{15}N$ and $\delta^{13}C$ stable isotopes, I aimed to determine the seasonal changes in diets and the isotopic niche spaces occupied by several common and abundant waterbird species in the lower reaches of the Kowie Estuary, South Africa. I wished to answer the following questions: 1) what resources do the waterbirds utilise 2) is there evidence of seasonal diet shifts in waterbirds in the Kowie Estuary, and 3) does each waterbird species occupy a unique isotopic niche? The following hypotheses were proposed: 1) each waterbird species utilises a unique prey resource each season 2) the diet of each waterbird species varies with seasonal resource availability, and 3) each species occupies unique trophic niche in each season.

3.2 Methods

3.2.1 Study Species

Five common waterbird species were selected for this study. All individuals were collected from the Kowie Estuary between June 2013 and May 2014 (Rhodes University ethics permit: ZOOL-09-2012, Department of Environmental Affairs permit: CRO 52/13CR and CRO 53/13CR, see Chapter 2 for site description and study map). The Cape Shoveller (*Anas smithii*, **Fig 4***)* is a common resident in South Africa, but is uncommon further north in Namibia, Botswana, Zimbabwe, Southern Angola, Lesotho, Mozambique and Zambia (Brown et al. 1982). Typically, Cape Shovellers weigh approximately 650 grams, and have a large black spatulate bill. They are gregarious when not breeding, and often form large flocks (Hockey et al. 2005). This duck is omnivorous, commonly consuming the stems and seeds of water plants, and dabbling in shallow water for snails, insects, molluscs, crustaceans and amphibian larvae (Brown et al. 1982, Hockey et al. 2005).

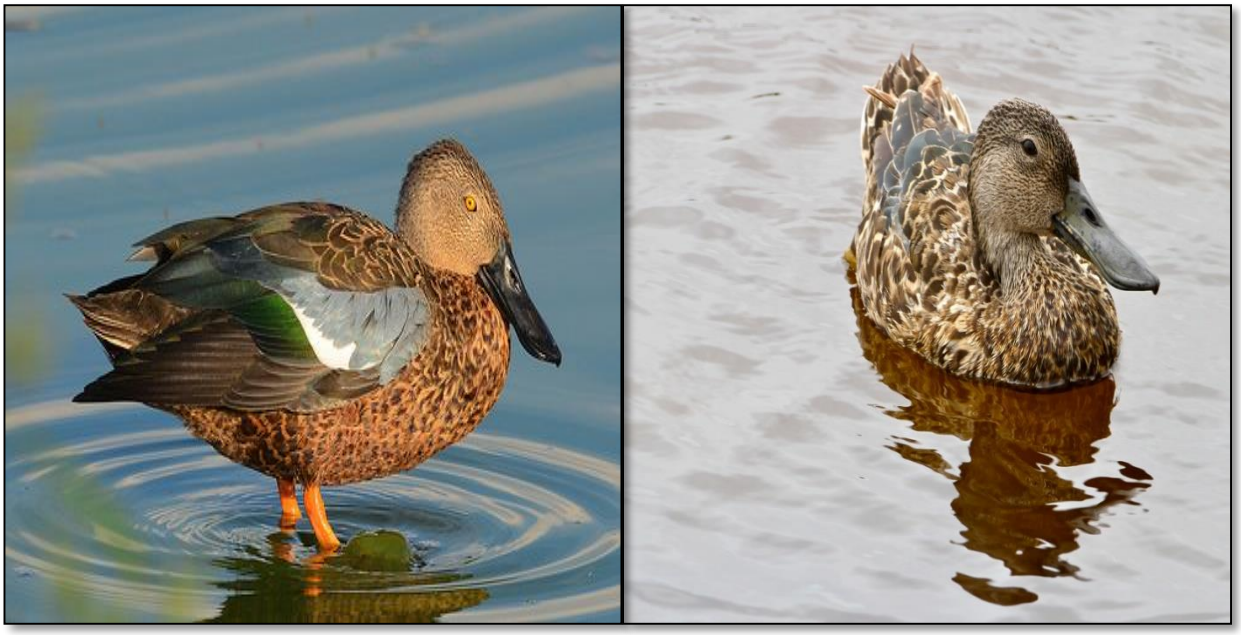

Fig 4: Adult male (left) and female (right) Cape Shoveller (*Anas smithii*) (White 2015).

The Cape Teal (*Anas capensis*, **Fig 5**) is a dabbling duck found in wetlands throughout sub-Saharan Africa (Brown et al. 1982, Hockey et al. 2005). While the species is essentially non-migratory, it can travel large distances (>100 km) to find suitable open water habitats (Brown et al. 1982, Hockey et al. 2005). The plumage of males and females is indistinguishable, with both sexes very pale and mainly grey in colour (Hockey et al. 2005). Cape Teal feed primarily on aquatic plants, crustaceans, annelids, chironomids and other small invertebrates (Hockey et al. 2005).

Fig 5: Adult (Male or female) Cape Teal (*Anas capensis*) (Ouzman 2015)

The Yellow-Billed Duck (*Anas undulata,* **Fig 6**) is a common resident of open waterways (including lagoons and estuaries), pans, lakes, dams and large rivers. This species is distinguishable from other duck species in the region by its bright yellow bill. Its diet is comprised primarily of aquatic vegetation (83%) and supplemented with invertebrates (mainly chironomid larvae) in winter (17%) (Hockey et al. 2005).

Fig 6: Indistinct plumage of adult male and female Yellow-Billed Duck (Clarence 2015)

The Ruff (*Phylomachus pugnax*, **Fig 7**) is a migrant Palearctic wader which displays marked sexual dimorphism (Brown et al. 1982). The male Ruff is much larger than the female Reeve, and has a breeding plumage that includes brightly coloured head tufts, bare orange facial skin, extensive black on the breast, and the large collar of ornamental feathers that inspired this bird's English name (Brown et al. 1982, Hockey et al. 2005).

Fig 7: Adult Ruff (*Phylomachus pugnax*) in non-breeding plumage (Burch 2015)

The Ruff forages in soft mud, probing or searching for visible items, taking insects, crustaceans, spiders, molluscs, worms, frogs, small fish, and occasionally feeding on the seeds of sedges, grasses and aquatic plants (Hockey et al. 2005). This highly gregarious bird is migratory and sometimes forms huge flocks on its non-breeding grounds, which include southern and western Europe, southern Africa, southern Asia and Australia (Hockey et al. 2005).

The Little Egret (*Egretta garzetta*, **Fig 8**) has a wingspan of approximately 88–106 cm long, and weighs 350–550 grams (Hockey et al. 2005). Its distribution ranges from Africa and Australia to Europe and Asia. In warmer locations, most birds are permanent residents (Brown et al. 1982). The diet of the Little Egret comprises of fish, insects, amphibians and crustaceans (Brown et al. 1982, Hockey et al. 2005).

Fig 8: Adult Little Egret (*Egretta garzetta*) (Jonczyk 2015)

3.2.2 Stable isotope analysis

Four to five individual birds per species were collected during winter (June - August 2013), spring (September - November 2013), summer (December 2013 - February 2014) and autumn (March - May 2014). Blood samples were collected immediately in the field and placed into 1 ml collection vials lined with Lithium Heparin (LASEC^{TM} mini-collect VGRV450478) on ice. Blood samples were separated into their constituent parts by spinning them at 6000 rpm for 15 min (LASEC^{TM} mini-centrifuge C1008-G). Blood cellular fraction and blood plasma were decanted into individual 1 ml blood collection vials, and stored at -80°C.

Frozen samples were freeze-dried (VirTis BenchTop 2K) at −60°C for 24 h, and subsequently ground into fine powder using lipid-cleaned pestle and mortars. To ensure that $\delta^{13}C$ and $\delta^{15}N$ stable isotope values were indicative of seasonal diet, the blood cellular fraction was used (hereafter referred to as "blood"). The blood cellular fraction of birds has a turnover rate of approximately one month (Hobson and Clark 1993), while blood plasma and whole blood have isotopic turnover rates of 12-24 h and 5-15 d respectively (Boecklen et al. 2011). The freeze dried samples were weighed into 8 × 5mm pressed tin capsules (OEA Laboratories LTD, Cornwall, UK). The $\delta^{13}C$ and $\delta^{15}N$ values of all samples were determined using a mass spectrometer with a Europa Scientific 20-20 IRMS linked to an ANCA SL prep unit. Beet sugar and ammonium sulphate were used as internal standards, and casein was used as a protein standard. Nitrogen was expressed (with analytical precision within +0.2‰) relative to atmospheric nitrogen, and carbon was expressed relative to Vienna Pee-Dee Belemnite. Isotope ratios are expressed in the δ unit notation in the following equation: $\Delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X represents $\delta^{13}C$ or $\delta^{15}N$ and R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio, respectively.

3.2.3 Data analysis

Kolmogorov-Smirnov tests of homogeneity revealed that all data were normally distributed ($p > 0.07$), and consequently, Analysis of Variance (ANOVA) was used to determine whether $\delta^{15}N$ and $\delta^{13}C$ stable isotope values were significantly different among seasons for each species. A mean value for $\delta^{15}N$ and $\delta^{13}C$ stable isotope values from all individuals of each species per season was calculated (referred to as a centroid), and thereafter multivariate analysis of variation (MANOVA) was used to test for inter-seasonal differences in the position of blood tissue centroids (in δ-space; Martínez del Rio et al. 2009). The $\delta^{15}N$ and $\delta^{13}C$ values of each individual per season was used in SIAR (stable isotope analysis in R - Bayesian mixing models) to determine the proportional contributions of selected diet items from the estuarine environment (Inger et al. 2013). Preliminary isoscapes were created each season using $\delta^{15}N$ and $\delta^{13}C$ values of all potential prey items (Bergamino 2014) and blood collected from waterbirds. Diet items were removed as potential prey items for each waterbird species in a stepwise manner according to dietary significance (e.g. fish are non-significant potential prey items for ducks). Consequently, the final choice of potential prey items used in the SIAR models for each species were chosen as "best fit" for that species by selecting potential prey based on current knowledge of waterbird diets. Potential prey items for each waterbird species were limited to five prey items because the accuracy of SIAR significantly decreases when more than five or six potential prey are used (Parnell and Jackson 2013).

Because of non-significant differences in the $\delta^{15}N$ and $\delta^{13}C$ of certain potential prey items, $\delta^{15}N$ and $\delta^{13}C$ values were pooled to create one unique prey item source value, i.e. *Hymensoma orbiculare* + *Sesarma catenata* = "crabs", and Amphipoda + Copepoda + crab zoea = micro-invertebrates, *Chenolea diffusa* + *Sarcocornia perennis* + *Phragmites australis* seeds = "salt marsh plants". While cord grass (*Spartina maritima*) is also considered to be a salt marsh plant, the $\delta^{15}N$ and $\delta^{13}C$ values obtained from *S. maritima* along the Kowie Estuary were significantly different to other salt marsh plants. As such, *S. maritima* and salt marsh plants were used as separate food sources in SIAR. The following potential prey items were used in SIAR models for; 1) Cape Shoveller (*Anas smithii*): salt marsh plants, *S. maritima*, micro-invertebrates, Mysidacea, *Palaemon peringueyi* and crabs, 2) Cape Teal (*Anas capensis*): micro-invertebrates, Mysidacea, *P. peringueyi* and crabs, 3) Yellow-Billed Duck (*Anas undulata*): micro-invertebrates, Mysidacea, *P. peringueyi*, and crabs, 4) Little Egret (*Egretta garzetta*): *Mugil cephalus*, *Liza dumerili, Sole bleekeri*, *P. peringueyi* and crabs, 5) Ruff: micro-invertebrates, Mysidacea and Isopoda. Tissue discrimination factors used per species were obtained from the literature, although there are no published values for the waterbird species used in this study. The following discrimination factors were used for a) all three duck species: $\delta^{15}N = 3.6 \pm 0.52$ ‰, $\delta^{13}C = -0.5 \pm 0.62$ ‰ (Bond and Jones 2009, Caut et al. 2009), b) Ruff: $\delta^{15}N = 2.91 \pm 0.0.16$ ‰, $\delta^{13}C = 1.15 \pm 0.18$ ‰ (Caut et al. 2009) and c) Little Egret: $\delta^{15}N = 4.0 \pm 0.2$ ‰, $\delta^{13}C = 2.0 \pm 0.2$ ‰ (Caut et al. 2009, Federer et al. 2010). Convex hulls were created using seasonal $\delta^{15}N$ and $\delta^{13}C$ stable isotope values for each species through the program SIBER (Stable Isotope Bayesian Ellipses in R), to calculate trophic niche in isotopic space (Layman et al. 2007, Jackson et al. 2011). Convex hull size and overlap of each species was calculated using SIBER, and statistically compared using ANOVA in R.

3.3 Results

3.3.1 Seasonal changes in $\delta^{15}N$ and $\delta^{13}C$ values of blood

With the exception of Ruff, all other species exhibited significant changes in $\delta^{15}N$ and $\delta^{13}C$ values among the seasons. There was a significant shift in the mean $\delta^{13}C$ value of Cape Shoveller from spring (-16.30 ± 2.52 ‰) to summer (-20.8 ± 6.8 ‰; $p = 0.011$), and from summer (-20.8 ± 6.8 ‰) to autumn (-17 ± 1.2 ‰; $p = 0.052$, **Fig. 9**). The mean $\delta^{15}N$ value of Cape Shoveller displayed a significant shift from spring (12.6 ± 1.5 ‰) to summer (8.5 ± 3.8 ‰; $p = 0.0001$), and from summer (8.5 ± 3.8‰) to autumn (13 ± 0.4‰; $p < 0.001$, **Figs. 9**).

Cape Teal exhibited a shift in $\delta^{13}C$ values from summer (-17.9 ± 2.1‰) to autumn (-15.2 ± 0.4‰) only ($p = 0.014$). Similarly, the $\delta^{15}N$ values of Cape Teal blood were significantly different when the seasons changed from summer (17.2 ± 4.2 ‰) to autumn (12.1 ± 0.6 ‰; $p < 0.001$, **Fig. 9**). Yellow-Billed Duck did not exhibit a significant shift in their $\delta^{13}C$ values among seasons, but did display a significant change in their $\delta^{15}N$ values between summer (17.3 ± 1.4 ‰) and autumn (8.7 ± 0.5 ‰; $p < 0.001$). The $\delta^{13}C$ values of Little Egret changed significantly from winter (-13.1 ± 0.2 ‰) to spring (-15.1 ± 0.7 ‰; $p < 0.001$), and from spring (-15.1 ± 0.7 ‰) to summer (-13.5 ± 0.4 ‰; $p < 0.001$, **Fig. 9**).

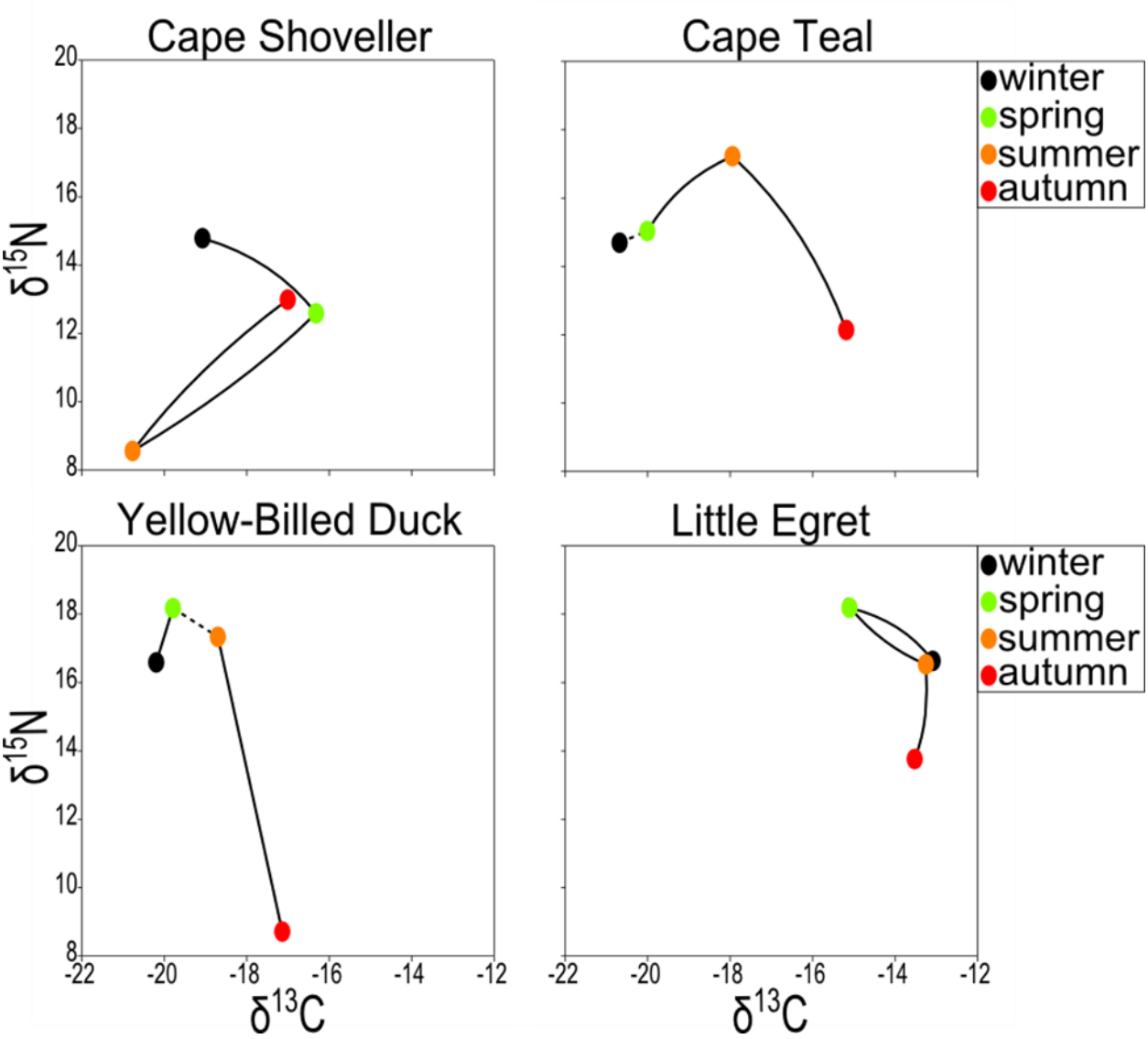

Fig 9: Differences in seasonal centroid positions of each waterbird species. Points represent mean values for blood tissue of each species per season (i.e. centroids). Solid lines connecting points indicates a significant shift in the position of centroid from one season to the next, dashed lines indicate non-significant seasonal shifts in centroid position.

The $\delta^{13}C$ and $\delta^{15}N$ values of blood cells in Ruff did not show any significant changes during the time that they spent in South Africa. Ruff was the only species that did not display a significant shift in centroid position. The centroid position of Cape Shoveller ($F = 12$, $p < 0.001$) and little Egret ($F = 111$, $p < 0.001$) exhibited a significant shift in position in δ-space every season (**Fig 9**). The centroid position of Cape Teal shifted significantly in δ-space from spring 2013 to summer 2014 ($p < 0.001$), as well as from summer 2014 to autumn 2014 ($p < 0.001$) only. Similarly, Yellow-Billed Duck displayed a significant shift in its centroid position from winter 2013 to spring 2013 ($p < 0.033$) and from summer 2014 to autumn 2014 ($p < 0.001$) only (**Fig 9**).

3.3.2 SIAR models

SIAR output showed how salt marsh plants and *S. maritima* steadily increased in prevalence in the diet of Cape Shoveller, from a micro-invertebrate dominated diet in winter 2013 (50%) to a diet that was purely herbivorous in summer 2013 / 2014. Salt marsh plants constituted 76% of the Cape Shoveller diet during the summer months, with *S. maritima* making up the remaining 24% of the summer diet (**Fig 10**). The diet of Cape Shoveller reverted to primarily feeding on micro-invertebrates (47%) during autumn 2014, with *S. maritima* still comprising 32% of the autumn diet (**Fig 10)**. Cape Teal displayed an increase in preference for crabs but a decreased preference for micro-invertebrates as the seasons progress from winter 2013 to autumn 2014 (**Fig 10**). Crabs constituted only 8% of the total diet in winter, increasing to 14% during the summer months until it finally became the dominant food source during the autumn months, constituting 73% of the diet of Cape Teal (**Fig 10**). Conversely, micro-invertebrates were the dominant food source in winter 2013, constituted 39% of the diet in winter but steadily decreased to only 8% in autumn 2014. Mysidacea were the dominant food source of Cape Teal in summer (31%) with *P. peringueyi* constituting 26% of the summer diet. Yellow-Billed Duck had a distinct preference for Mysidacea during winter (71%), spring (57%) and summer (47%). But while Mysidacea decreased in prevalence in the diet of Yellow–Billed Duck from winter 2013 to autumn 2014, the prevalence of micro-invertebrates slowly increased and became the dominant food source in autumn 2014 (34%) (**Fig 10**). SIAR models for Little Egret revealed that flathead mullet (*M. cephalus*) was the dominant food source across all seasons (30% - 91%), but is particularly dominant in the Little Egret diet during winter (91%) and summer (86%). The striped mullet (*L. dumerili*) became more prevalent in the diet of Little Egret in spring (23%), as did *S. bleekeri* (19%) (**Fig 10**).

Little Egret incorporated crabs into their diet during the autumn months of 2014, with crabs making up 21% of the autumn diet. Micro-invertebrates were the dominant food source for Ruff in both the spring (70%) and summer months (65%) (**Fig 10**) in South Africa.

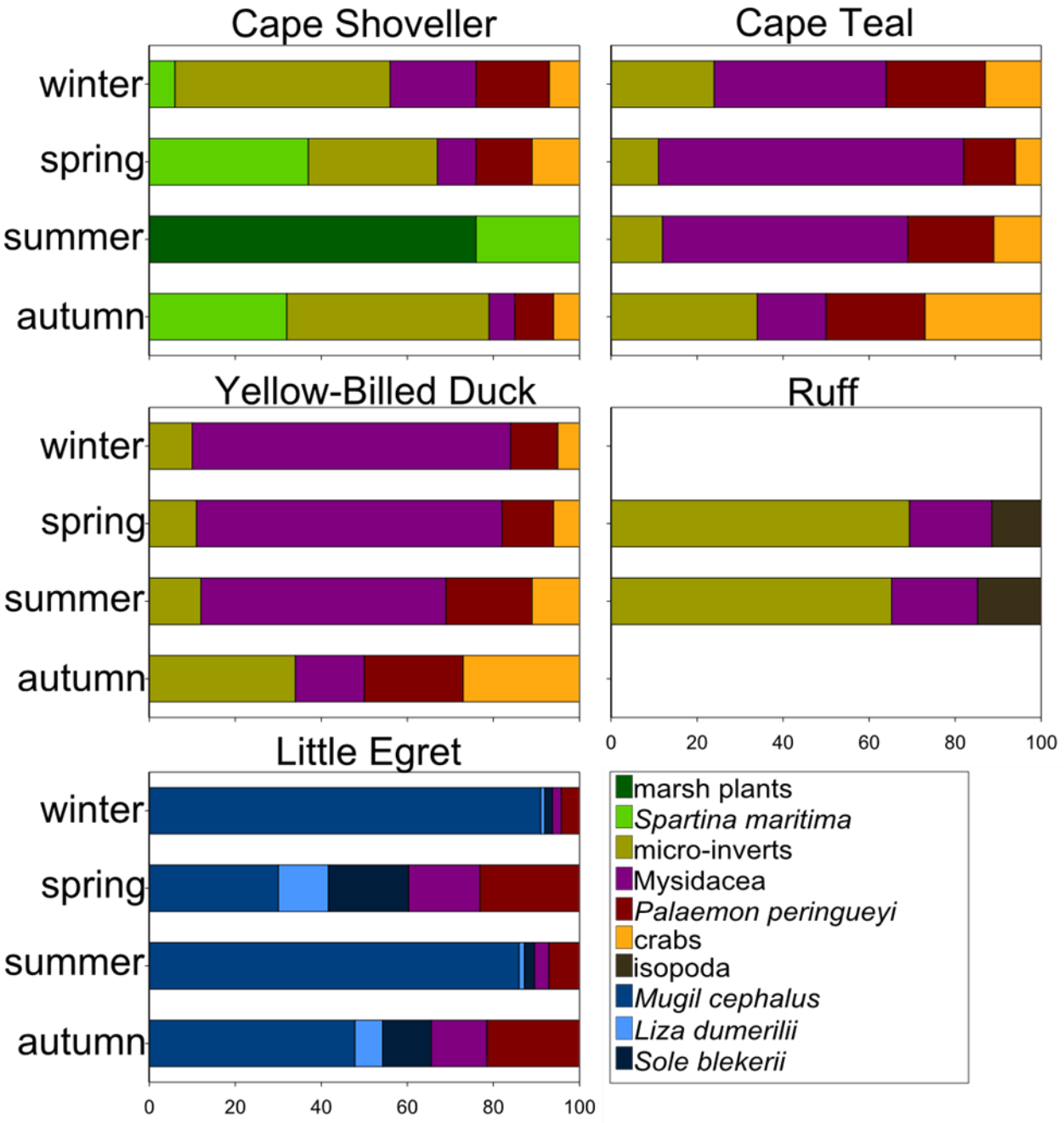

Fig 10: SIAR mixing model output for Cape Shoveller (*Anas smithii*), Cape Teal (*Anas capensis*), Yellow-Billed Duck (*Anas undulata*), Little Egret (*Egretta garzetta*) and Ruff (*Philomachus pugnax*). Prey items are presented as calculated percentage of total diet per season (x-axis).

3.3.3 Isotopic niche

Cape Shoveller (df = 3, F = 97, p < 0.001), Cape Teal (df = 3, F = 14, p < 0.001) and little Egret (df = 3, F = 5, p = 0.021) displayed significant seasonal changes in their isotopic niche widths, while the isotopic niche widths of Yellow-Billed Duck (F = 0.35, p = 0.790) and Ruff (t = 0.97, p = 0.359) did not exhibit any significant temporal variation (**Fig 11**). The niche width of Cape Shoveller was largest in summer, whereby the summer niche was significantly larger than those in winter (p < 0.001), spring (p < 0.001) and autumn (p < 0.001), while the isotopic niche width of Cape Shoveller in spring was significantly larger than that in autumn (p = 0.025). Similarly, the isotopic niche width of Cape Teal in summer was significantly larger than the winter (p = 0.003), spring (p=0.002) and autumn (p < 0.001, **Fig 11**). Little Egret displayed a significant expansion of its isotopic niche from winter to spring (p = 0.012), and then a significant contraction from spring to summer (p = 0.050, **Fig 11**).

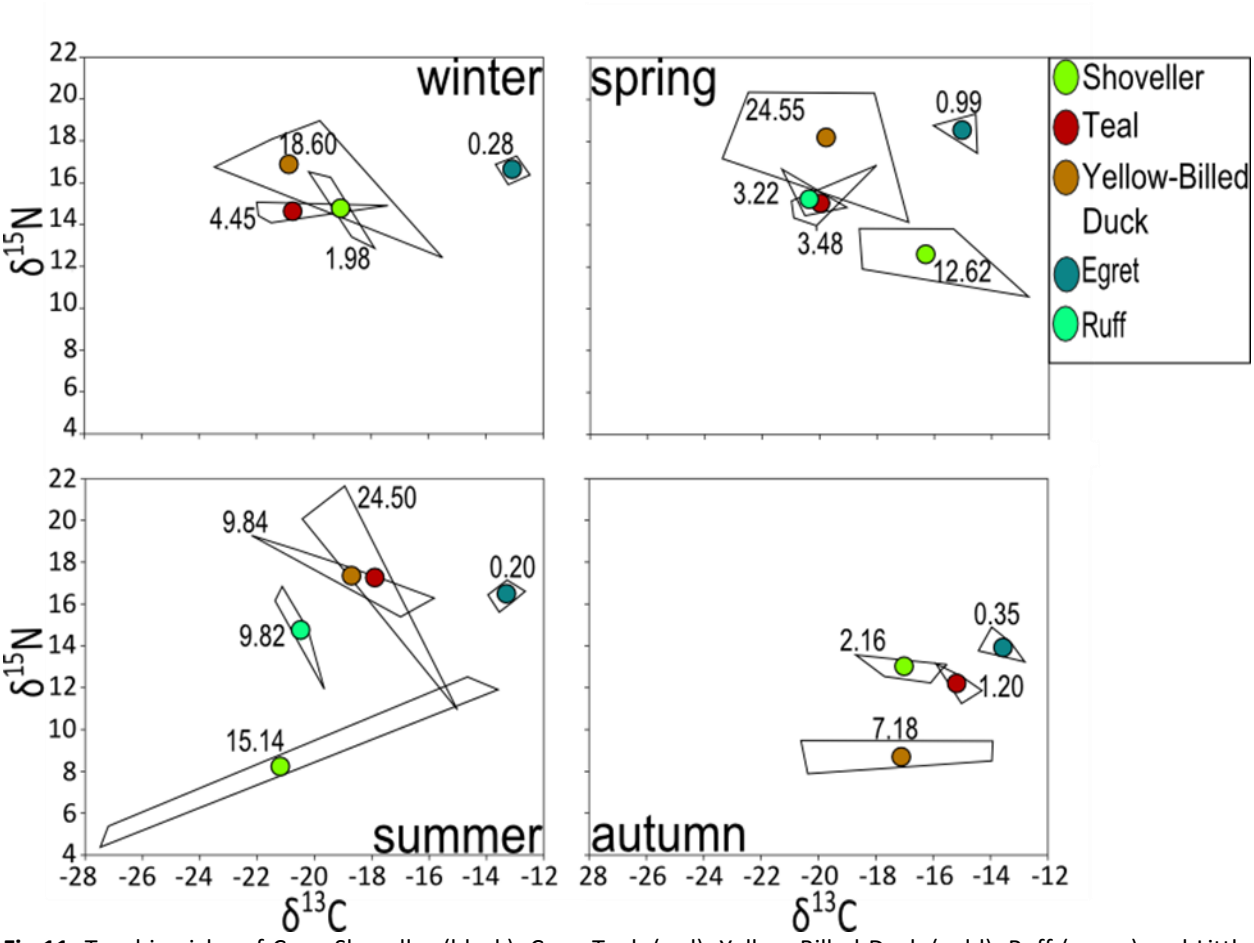

Fig 11: Trophic niche of Cape Shoveller (black), Cape Teal, (red), Yellow-Billed Duck (gold), Ruff (green) and Little Egret (blue) during winter 2013, spring 2013, summer 2014 and autumn 2014. Large solid colour circles represent the centroid of each convex hull. Values closest to each convex hull represent isotopic niche width.

The isotopic niches of Cape Teal and Yellow-Billed Duck overlapped during winter (52%), spring (62%) and summer (61%). The isotopic niches of Cape Shoveller and Yellow-Billed Duck overlapped by 67% during winter, while Ruff and Cape Teal shared 62% of their isotopic niche space during the spring (**Fig 11**).

3.4 Discussion

This study aimed to determine resource use by waterbirds in the Kowie Estuary, seasonal diet shifts, and the trophic niches of each species. The stable isotope data revealed that waterbirds utilised several abundant resources in the Kowie Estuary. Similarly, stable isotope techniques revealed seasonal diet shifts and seasonal variations in the trophic niche of each species. The first hypothesis, that waterbirds along the Kowie Estuary use different prey resources, was supported. The second hypothesis, that each species displays seasonal diet shifts as revealed by stable isotope analysis, was also supported. Resource availability within the mudflats and dietary requirements may have underpinned the stochastic nature of waterbird diet between seasons. Data extrapolated from Heyns & Froneman (2010) and Bergamino & Richoux (2014) on relative prey abundance revealed that micro-invertebrates (as defined in the methods section) was the most abundant food source in the Kowie Estuary across all seasons. Micro-invertebrate abundance peaks during the summer and are 20 times more abundant than other food sources in the estuary. The relatively high abundance of micro-invertebrates during winter (only 38% lower than the summer abundance) could explain why micro-invertebrates comprised a relatively large portion of Cape Shoveller and Cape Teal during winter. Micro-invertebrate abundance in spring was significantly lower compared to winter (68% reduction), and the proportion of micro-invertebrates in the diet of Cape Shoveller and Cape Teal diet reflected this. However, the arrival of large numbers of Ruff to the Kowie Estuary in early to mid-spring may also account for the reduced intake of micro-invertebrates by Cape Shoveller during spring and summer. Cape Shoveller made a distinct shift to a vegetative diet upon the arrival of Ruff to the Kowie mudflats (personal observation 2013, 2014). The relative abundance of Mysidacea in the Kowie Estuary was highest during spring, with the relative abundance of *P. peringueyi* in the Kowie Estuary being highest during summer (Bergamino and Richoux 2014). SIAR models revealed that Cape Teal and Yellow-Billed Duck take advantage of these relative increases in prey abundance by increasing their intake of Mysidacea during spring and *P. peringueyi* during summer. Cape Shoveller and Yellow-Billed Duck incorporated more micro-invertebrates into their diet during autumn, despite a typical 45% reduction in the relative abundance of micro-invertebrates in autumn compared to summer (Heyns and Froneman 2010).

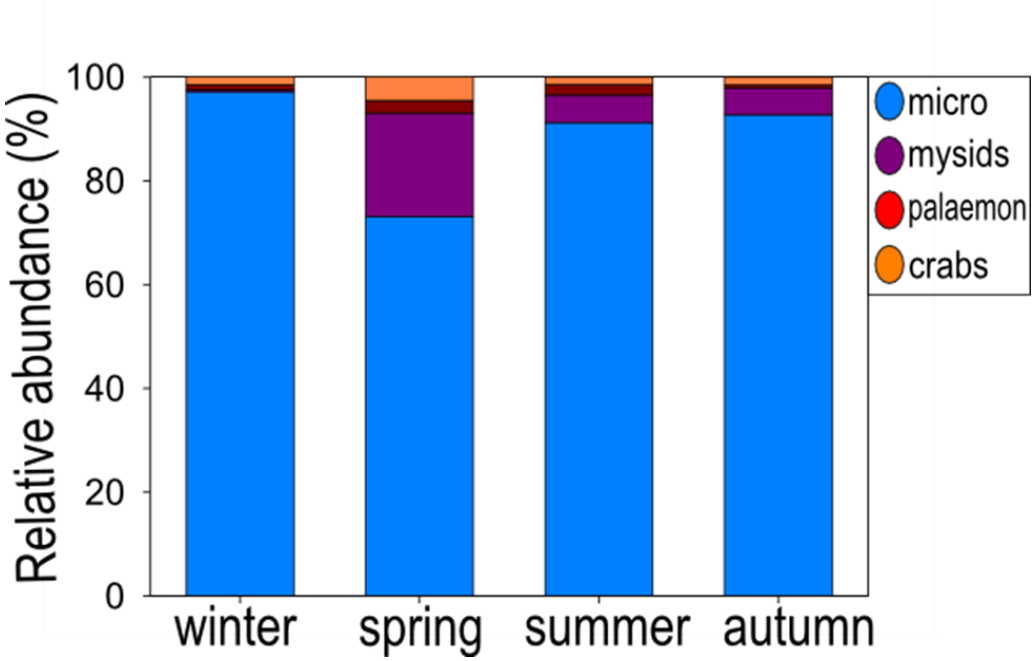

Fig 12: Relative abundance of micro-invertebrates (micro), Mysidacea (mysids), *P. peringueyi* (palaemon) and crabs along the Kowie Estuary (Heyns and Froneman 2010, Bergamino and Richoux 2014).

Data on the ichthyofauna that occupy warm-temperate estuaries were extrapolated from Harrison and Whitfield (2006). Of the three fish species used in the reconstruction of Little Egret diet (i.e. *L. dumerili, M. cephalus and S. bleekeri),* striped mullet (*L. dumerili*) had the highest relative abundance of the three species, but *M. cephalus* (flathead mullet) had the highest relative biomass. The typical elevated biomass of *M. cephalus* may explain why Little Egret preferentially fed on this fish species throughout the year in the Kowie system. Juvenile *M. cephalus* occupy shallow back-waters in estuarine habitats (Blaber and Whitfield 1977), a preferred foraging ground of Little Egret in the Kowie Estuary (personal observations 2013, 2014). The fluctuations in the proportion of each potential fish prey item to the diet of little Egret may be associated with seasonal fluctuations in prey abundance. Conversely, the increased intake of crabs into the Little Egret diet during spring and autumn was contrary to the seasonal peak abundance of crabs during winter and summer (Bergamino and Richoux 2014), but may illustrate the opportunistic intake of an additional protein source during periods of low *M. cephalus* abundance. There was evidence of overlap amongst the isotopic niches of waterbirds along the Kowie Estuary, and there appeared to be preferences for similar food resources amongst all three duck species and Ruff. Consequently, I rejected the third hypothesis that the each waterbird species has a unique trophic niche during each season. The large amount of overlap between the convex hulls of Cape Teal and Yellow-Billed Duck during winter, spring and autumn suggested potential for interspecific competition between these two species.

Cape Teal and Ruff occupied the same isotopic niche space during spring, possibly indicating interspecific competition for resources between these two species. Despite a decrease in resource availability during autumn (Heyns and Froneman 2010, Bergamino and Richoux 2014), it was the only season in which each species occupied a unique niche space. The small sample size in this instance must be taken into account, and the data may not fully represent the trophic interactions of these species nor truly reflect the seasonal dietary range of these waterbird species. Additionally, because waterbirds undoubtedly travel between the Kowie Estuary and neighbouring estuarine ecosystems such as the nearby Kariega Estuary and Bushman's Estuary, the waterbird species that I have used in my study will, in all likelihood, be ingesting prey items from estuaries other than the Kowie Estuary, which may skew the interpretation of their diet. The movement of waterbirds between foraging patches and/or neighbouring ecosystems is uncontrollable, but is a caveat that must be seriously considered. A literature search revealed that prey items collected from the Kowie Estuary have similar isotopic values to prey items in neighbouring ecosystems (see Richoux and Froneman 2009), therefore significantly reducing the margin of error in diet determination inferred by waterbird movement. Consequently, these data suggest that the SIAR models used in this study to determine waterbird diet may be accurate.

When preferred resources are scarce, individuals expand their feeding niche to accept previously unutilised or under-utilised resources (Hammerschlag-Peyer et al. 2011). An example of diet switching to underutilised resources was when Cape Shoveller shifted from feeding on nutrient and protein-rich micro-invertebrates in autumn and winter to salt marsh plants and cord grass (*S. maritima*) during spring and summer. The niche width of a species may depend on the diversity of resources that are available to individuals (also referred to as "ecological opportunity", Araújo et al. 2011) and the overall abundance of a particular resource (Bolnick et al. 2003, 2011, Araújo et al. 2011). Terrestrially-based consumers that feed in aquatic habitats, such as waterbirds which feed across multiple trophic levels, can significantly alter trophic pathways and energy fluxes in the habitats in which they feed through their diet switching (Polis and Strong 1996, Shaner and Macko 2011). Previous studies that have investigated consumer diets have used trophic shifts and diet switching as indicators of perturbations in food webs, such as spatial or temporal subsidies into an ecosystem (e.g. Stapp et al. 1999, Vander Zanden and Rasmussen 1999). A seasonal increase of a resource can have broad effects on species interactions (Ostfeld and Keesing 2000, Yang et al. 2008). Consumers that occupy upper trophic levels can often be opportunistic foragers, and they are expected to respond quickly to resource pulses, both numerically and functionally (Sears et al. 2004).

A decrease in the availability of a particular resource over time, coupled with an increase in the consumer population (e.g. arrival of numerous Ruff in the spring remaining until early autumn), often leads to diet switching by consumers to alternative prey, resulting in complex trophic interactions amongst and within species (Ostfeld and Keesing 2000, Schmidt and Ostfeld 2003). Variations in seasonal diet and resource partitioning amongst co-occurring species has occurred in different groups, including seabirds (e.g. Cherel et al. 2008, Jaeger et al. 2009, 2010), marine mammals (e.g. Gavrilchuk et al. 2014) and marine reptiles (e.g. Vander Zanden et al. 2010, Thomson et al. 2012) but there is little literature highlighting the seasonal diet shifts of waterbirds. Karnovsky et al. (2008) discovered that the diet of three seabird species, Dovekie, Black-legged Kittiwake and Thick-billed Murre, displayed significant seasonal shifts that coincided with seasonal prey abundances (also see Hedd et al., 2010).

Investigations that wish to reconstruct the diet of waterbirds need to consider the isotopic half-life of tissues and the season in which the tissue is sampled. Waterbirds appear to shift their diet rapidly in response to resource availability and may be linked to interspecific competition with other waterbird species. Consequently, the predatory pressure that waterbirds exert on aquatic food webs, and the prey communities in them, have a strong seasonal effect. Furthermore, scientists need to take into account that waterbirds, despite being described as generalist feeders, may actually exhibit short-term specialist-type feeding behaviour, particularly when a single type of food is abundant. Mysidacea can swarm in vast numbers in estuaries in the Eastern Cape of South Africa during the spring (personal observations 2013, 2014). In these instances, waterbirds will no doubt take advantage of this plentiful food resource, or any other on hand that is abundant in the short-term. Cape Teal displayed an increase in their consumption of crabs with an increase in crab abundance along the Kowie Estuary mudflats. Similarly, there was a marked increase in the consumption of Myscidacea by Yellow-Billed Duck as the abundance of Myscidacea increased in the Kowie Estuary. The role that waterbirds play in the energy fluxes of estuaries is complex. Presently, we lack a means to accurately characterise the trophic niches of higher-order animals, so that food web ecologists are forced to consolidate species that occupy the upper trophic levels of food webs into broad trophic subsets, such as carnivore, omnivore, piscivore or insectivore (Steffan et al. 2013). There is clearly a need for greater resolution in the measurement of trophic attributes (Sih et al. 1985, 1998, Polis and Strong 1996, Steffan et al. 2013). Relegating species to coarse-grain classifications effectively overlooks vertical trophic diversity, and lumps together omnivore and carnivore groups that may have contrasting impacts on primary production and/or prey items lower down the food web (Duffy et al. 2007, Estes et al. 2011, Steffan et al. 2013).

Stable isotope analysis of multiple body tissues reveals intermittent diet switching in three estuarine ducks.

4.1 Introduction

Consumers are key regulators of prey communities, particularly in aquatic ecosystems, where top-down trophic cascades can be initiated through variations in consumer abundance (Leroux and Loreau 2008, Matich et al. 2011). Generalist consumers, particularly seabirds and waterbirds that feed across trophic levels, can alter trophic flows in aquatic food webs through their diet switching (Polis and Strong 1996, Post 2002). Tracking the diets of aquatic-feeding birds is critical if we are understand the role that they play as consumers in aquatic food webs. The determination of bird diets over long periods allows scientists to map variations in resource use, which ultimately provides insight into perturbations in prey community abundance and occurrence. While non-destructive tissue sampling (e.g. blood or feather sampling) for use in stable isotope based diet determination is of immense use, this technique is subject to inherent biases. The use of blood and feather samples alone in diet determination may not account for variations in diet over short- and intermediate time frames. For example, flight feathers provide information on food ingested when feathers were grown (Hobson and Clark 1992b, Boecklen et al. 2011). Likewise, blood tissue samples (either whole blood or constituent parts) provides information on recently assimilated food. In many instances, the time frame that separates the sampling of these two tissues may be several months apart. Nevertheless, there are numerous studies that have utilised this approach to investigate several aspects of seabird and waterbird trophic ecology (e.g. Martínez del Rio et al. 2009, Collier and Lyon 2010, Phillips et al. 2011, Tilley et al. 2013).

Researchers have utilised more than one tissue type to examine bird diets and trophic niche switching (e.g. Shaner and Macko 2011), seabird diets during and outside the breeding seasons (e.g. Hebert et al. 2009, Young et al. 2010, Catry et al. 2014), migratory patterns (e.g. Fraser et al. 2008, González-Solís et al. 2011, Carlisle et al. 2012), and intra- and interspecific competition for food resources (Sabat and Martínez del Rio 2002, Herrera et al. 2003, Cherel et al. 2007, Connan et al. 2007, Jaeger et al. 2009, Martínez del Rio et al. 2009). A thorough search of the literature revealed that there were no published studies that utilised multiple (i.e. > 2) body tissues to determine the diets of seabirds or waterbirds. Investigations into the isotopic composition of several body tissues provides an opportunity to determine the foraging and trophic ecology of seabirds and waterbirds over several temporal scales (Hobson and Clark 1992b, Dalerum and Angerbjörn 2005a, Bolnick et al. 2011, Araújo et al. 2011).

Additionally, the use of several body tissues with dissimilar isotopic half-lives may provide insight into whether the diet of waterbirds is highly variable or relatively consistent over time. Certain tissues, such as liver and blood plasma have high turnover rates, and their isotopic composition reflect diet items integrated recently (hours to 2 weeks), while tissues such as bone collagen and claws reflect dietary items incorporated over extended periods (6-12 months; Boecklen et al. 2011). Inert tissues such as primary flight feathers and down feathers are deposited in a relatively short and discrete time interval, and their isotopic composition reflects dietary resources incorporated while the tissue was manufactured (e.g. Hobson and Clark 1992b, Boecklen et al. 2011, Hobson 2011, Bearhop et al. 2013). With the variation of isotopic half-lives taken into account, the isotopic analysis of various tissues from a group of individuals allows the breadth of the isotopic niche to be inferred at different temporal scales (Shaner and Macko 2011). The aim of this study was to determine: 1) if the isotopic composition differs amongst body tissues having variable turnover times (see **Table 5**), 2) the inter- and intra-seasonal changes in the diets of several waterbird species, and 3) the inter- and intra-seasonal variability in isotopic niche of each waterbird species. The following hypotheses were tested: 1) each species exhibits inter-seasonal diet shifts (i.e. differences in the isotopic composition of inert / slow turnover tissues), but not intra-seasonal diet shifts (i.e. no difference in the isotopic composition of quick turnover tissues), and 2) the isotopic niche of each species varies over time as each species feeds upon dissimilar prey items each season.

4.2 Methods

4.2.1 Sample collection

Primary flight feathers, down feathers, claws, muscle tissue and liver tissue were sampled from Cape Shoveller (*Anas smithii*), Cape Teal (*Anas capensis*), and Yellow-Billed Duck (*Anas undulata*) (Rhodes University ethics permit: ZOOL-09-2012, Department of Environmental Affairs permit: CRO 52/13CR and CRO 53/13CR, see Chapter 2 for site description and Chapter 3 for species descriptions). These three duck species are common and abundant in the lower reaches of the Kowie Estuary, and they forage on the mudflats year round. Four to five individual per species were collected during winter (June-August 2013), spring (September-November 2013), summer (December 2013- February 2014) and autumn (March-May 2014). Primary flight feathers 1 and 2 were taken from the right wing of each bird and several down feathers were taken from the pectoral region. Pectoral muscle and liver tissue samples were placed into ashed (450°C muffle furnace for 4 hours) tin foil envelopes and stored at -80°C.

Frozen muscle and liver samples were freeze-dried at −60°C for 24 h (VirTis BenchTop 2K), and subsequently ground into fine powder using lipid-cleaned pestle and mortars. All feather, claw, muscle and liver samples were placed into 15ml glass vials and cleaned of lipids by rinsing them in a 5 ml of 2:1 (chloroform: methanol + 1 ml deionised water) for 24 hours (Post et al. 2007a). Blood samples were prepared according to Chapter 3. All samples were dried at 50°C for 24 h and analysed for $\delta^{15}N$ and $\delta^{13}C$ stable isotopes following the methods outlined in Chapter 3.

4.2.2 Data analysis

To investigate temporal variation in diet, differences in the $\delta^{15}N$ and $\delta^{13}C$ values of tissues from each species were tested using several statistical tests. Kolmogorov-Smirnov tests of homogeneity revealed that all data were normally distributed ($p > 0.05$). Multivariate analysis of variation (MANOVA) was used to test for intra-seasonal differences in the position of centroids among tissues (in δ-space) (Martínez del Rio et al. 2009). Secondly, SIAR models were run using each tissue type collected from each duck population to determine diet using diet items collected each season (Bergamino and Richoux 2014). Preliminary isoscapes were created each season using $\delta^{15}N$ and $\delta^{13}C$ values of all potential prey items (Bergamino 2014) and blood collected from waterbirds. Diet items were removed as potential prey items for each waterbird species in a stepwise manner according to dietary significance (e.g. fish are non-significant potential prey items for ducks). Consequently, the final choice of potential prey items used in the SIAR models for each species were chosen as "best fit" for that species by selecting potential prey based on current knowledge of waterbird diets. Potential prey items for each waterbird species were limited to five prey items because the accuracy of SIAR significantly decreases when more than five or six potential prey are used (Parnell and Jackson 2013). The following potential prey items were incorporated: 1) for Cape Shoveller (*Anas smithii*) and Cape Teal (*Anas capensis*): salt marsh plants, *S. maritima*, micro-invertebrates, Mysidacea, *Palaemon peringueyi* and crabs, 2) for Yellow-Billed Duck (*Anas undulata*): *S. maritima, Codium spp,* micro-invertebrates, Mysidacea, *P. peringueyi*, and crabs. See Chapter 3 for definitions of "micro-invertebrates" and "crabs". Cord grass (*S. maritima*) is considered as a salt marsh plant, but the $\delta^{15}N$ and $\delta^{13}C$ values obtained from *S. maritima* along the Kowie Estuary were significantly different to other salt marsh plants. Subsequently, *S. maritima* and salt marsh plants were used as separate food sources in SIAR. The isotopic turn-over rate of tissues were sourced from the literature (**Table 5**), as well as the tissue discrimination factors (Caut et al. 2009, Hahn et al. 2012) used in SIAR models because there is no current information for these specific waterbird species used in this study.

Thus, the following discrimination factors were used for each tissue in SIAR model to determine diet; primary feathers: $\delta^{15}N = 5.2 \pm 0.56$ ‰, $\delta^{13}C = 0.9 \pm 0.73$ ‰, pectoral feathers: $\delta^{15}N = 4.7 \pm 0.71$ ‰, $\delta^{13}C = 1.0 \pm 1.03$ ‰, claws: $\delta^{15}N = 4.5 \pm 0.67$ ‰, $\delta^{13}C = 0.4 \pm 0.58$ ‰, cellular blood: $\delta^{15}N = 3.6 \pm 0.52$ ‰, $\delta^{13}C = -0.50 \pm 0.62$ ‰, muscle: $\delta^{15}N = 1.70 \pm 0.43$ ‰, $\delta^{13}C = 0.92 \pm 0.27$ ‰ and liver: $\delta^{15}N = 3.84 \pm 0.26$ ‰, $\delta^{13}C = 0.35 \pm 0.32$ ‰. Isotopic niche width was used as a measure of niche space (Layman et al. 2007, but see Chapter 3 for full description), and intra-seasonal differences in isotopic niche width were assessed using ANOVA. All statistical analyses were performed in R including SIAR (Windows 7) and all graphics were produced using INKscape™.

Table 5: Time span of dietary intake that each tissue stable isotope composition represents.

Tissue type	Isotopic turnover rate	Reference
flight feathers	180 – 360 d	Cherel et al. 2000, Forero and Hobson 2003
down feathers	120 - 160 d	Cherel et al. 2000, Forero and Hobson 2003
claws	120 d	Boecklen et al. 2011
blood	30 d	Hobson and Clark 1992, Boecklen et al. 2011
pectoral muscle	25 d	Hobson and Clark 1992, Boecklen et al. 2011
liver	5 d	Hobson and Clark 1992, Boecklen et al. 2011

4.3 Results

4.3.1 Cape Shoveller (*Anas smithii*)

4.3.1.1 Seasonal tissue differences ($\delta^{15}N$ & $\delta^{13}C$)

All tissues collected from Cape Shoveller in winter ($F_3 = 18$, $p < 0.001$), spring ($F_3 = 52$, $p < 0.001$), summer ($F_3 = 21$, $p < 0.001$), and autumn ($F_3 = 22$, $p < 0.001$) displayed shifts in the positions of centroids (**Fig. 13**). Cape Shoveller individuals collected from each season exhibited changes in their trophic level with season. Isotopic signatures in flight feathers (which represent the diet in the most distant past), showed that Cape Shoveller was a primary consumer, but liver tissue (which represents the very recent diet) indicated a high trophic level for all individuals (see **Fig 13**). Cape Shoveller individuals steadily shifted their diet and increasingly fed on food resources higher up the trophic levels as the seasons progressed.

The significant difference in the positions of each tissue centroid ($p < 0.001$ in all instances) revealed that Cape Shoveller exhibit both inter- and intra-seasonal diet shifts, particularly evident from tissues collected in winter, spring and summer (**Fig 13**). Samples collected from individuals in autumn 2014 displayed some stability in Cape Shoveller diet, as signified by claw tissue, blood tissue and muscle tissue

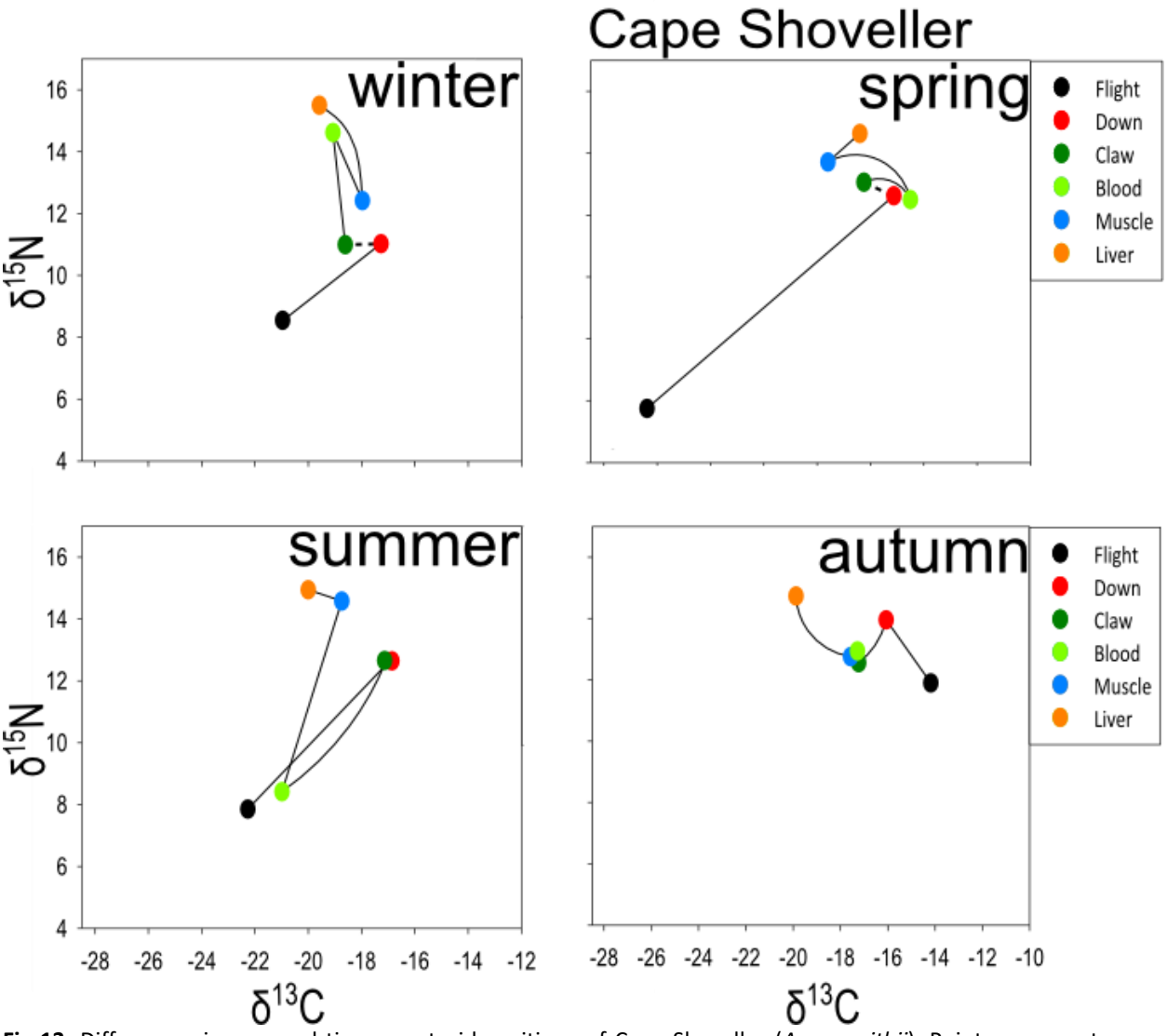

Fig 13: Differences in seasonal tissue centroid positions of Cape Shoveller (*Anas smithii*). Points represent mean values for tissue type (i.e. centroids). Lines connecting points indicated significant difference in δ-space position. Abbreviations: Flight = primary flight feathers, Down = pectoral down feathers.

4.3.1.2 SIAR models

Cape Shoveller collected in winter and spring of 2013 exhibited purely vegetative diets occurred in late winter / early spring 2012, as shown by SIAR models for flight feathers. Flight feathers collected in summer suggested a similar vegetative diet in late winter / early spring 2013, while flight feathers from individuals in autumn 2014 showed increase incorporation invertebrates into Cape Shoveller diets (**Table 6**, **Fig 14**). There was a large shift in their diet from spring 2012 (flight feathers) to autumn 2013 (down feathers and claws), when Mysidacea (32%) and micro-invertebrates (22%) constituted the largest proportion of the birds' diet. Further into winter, the diet of Cape Shoveller remained relatively constant, but there was an increase in the proportion of micro-invertebrates in their diet from autumn 2013 to winter 2014 (**Table 6, Fig 14**). *S. maritima* (5% - 12%) and crabs (7% - 11%) constituted the smallest proportion of the birds' diet during winter. SIAR models for tissues collected in spring 2013 showed that the diet of Cape Shoveller was comprised primarily of micro-invertebrates during winter 2013 and spring 2013, with a slight increase in the prevalence of *S. maritima* in spring (see blood tissue, **Fig 14**). Additionally, as the spring progressed, Cape Shoveller incorporated more Mysidacea and *P. peringueyi* into its diet (see liver tissue, **Fig 14**). Cape Shoveller diet displayed the largest shift during the summer. The diet switched from a purely vegetative diet to an invertebrate dominated diet in spring (see change in flight feathers to down feathers and claws, **Fig 14**), similar to the diet of individuals collected in spring. SIAR models revealed that Cape Shoveller diets were primarily herbivorous during early to mid-summer, but Cape Shoveller exhibited an increase in the incorporation of micro-invertebrates into their diet during late summer (47% - 58%; Table 6, **Fig 14**). The diet of Cape Shovellers collected in autumn 2014 displayed an increase in the prevalence of micro-invertebrates in their diet from winter 2013 through to autumn 2014. *S. maritima* constituted the largest amount to Cape Shoveller diet in late winter 2013 (see flight feathers, **Fig 14**) but decreased in summer (see down feathers and claws) and into autumn (see blood, muscle and liver tissue, **Table 6**, **Fig 14**).

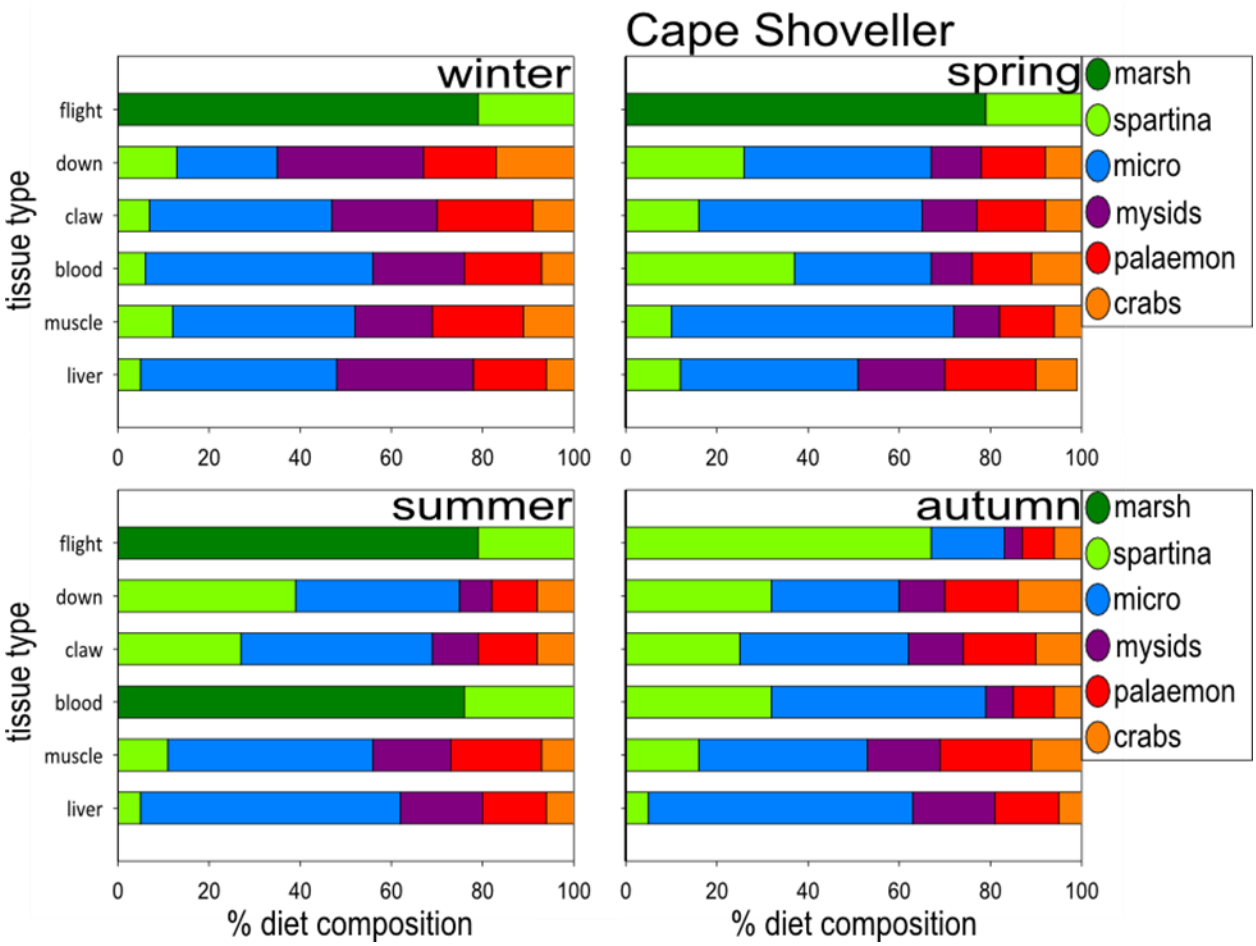

Fig 14: SIAR mixing model output for each tissue type sampled from Cape Shoveller collected across four seasons. Horizontal bars represent percentage (%) contribution of prey items to overall diet. Abbreviations: marsh = marsh plants, spartina = *S. maritima*, micro = micro-invertebrates, mysids = Mysidacea, palaemon = *P. peringueyi*. Tissues are listed in descending order of tissue turn-over rate (i.e. flight > down > claw > blood > muscle > liver).

4.3.1.3 Isotopic niche

The isotopic niches depicted by the different tissues varied through time (**Fig 15**). The niche width denoted by flight feathers from winter, spring and summer was significantly larger than those of all other tissues in those seasons ($p < 0.001$ in all instances). The niche width of Cape Shoveller significantly contracted from winter 2012 (14.9‰) to autumn 2013 (3.2‰ – 2.5‰) (see down feathers and claws, **Fig 15**). There was a large overlap (76%) between niches depicted from down feathers and claws in winter (**Table 7**, **Fig 15**).

The convex hull depicted by flight feathers overlapped with that of down feathers by 14%, while the convex hull depicted by blood overlapped with that of liver tissue by 37%. Flight feathers (i.e. winter 2012, 4.8‰) and blood tissue (4.7‰) produced the largest niche widths (**Table 7**, **Fig 15**). The niche widths depicted by down feathers and claws were significantly smaller than that of flight feathers. The niche width from blood tissue, representing the spring 2013 niche, increased in size from winter (see down feathers and claws; see **Fig 15**). The isotopic niches derived from of muscle (1.3‰) and liver tissue (0.8‰) gradually decreased in size as the spring season progressed (see **Table 7**). The isotopic niches derived from tissues divided roughly into two groups, similar to spring.

The niche width during winter 2013 (see flight feathers) was significantly larger than that during spring (see down feathers and claws, **Fig 15**), with the isotopic niche width of summer sampled individuals rapidly contracting in size. Conversely, the isotopic niche of blood tissue (i.e. the summer 2013 niche) was significantly larger than all other tissue types, with the exception of flight feathers (Fig 15). The niche width of individuals in summer subsequently contracted in size (see muscle and liver tissue, **Table 7**, **Fig 15**). Cape Shoveller individuals collected in autumn exhibited uniquely small isotopic niches, whereby the niche width represented by each tissue type was not significantly different to one another ($F_5 = 0.916$, $p = 0.497$). The convex hulls depicted by claws, blood and muscle occupied the same isotopic space and overlapped by 50-80%.

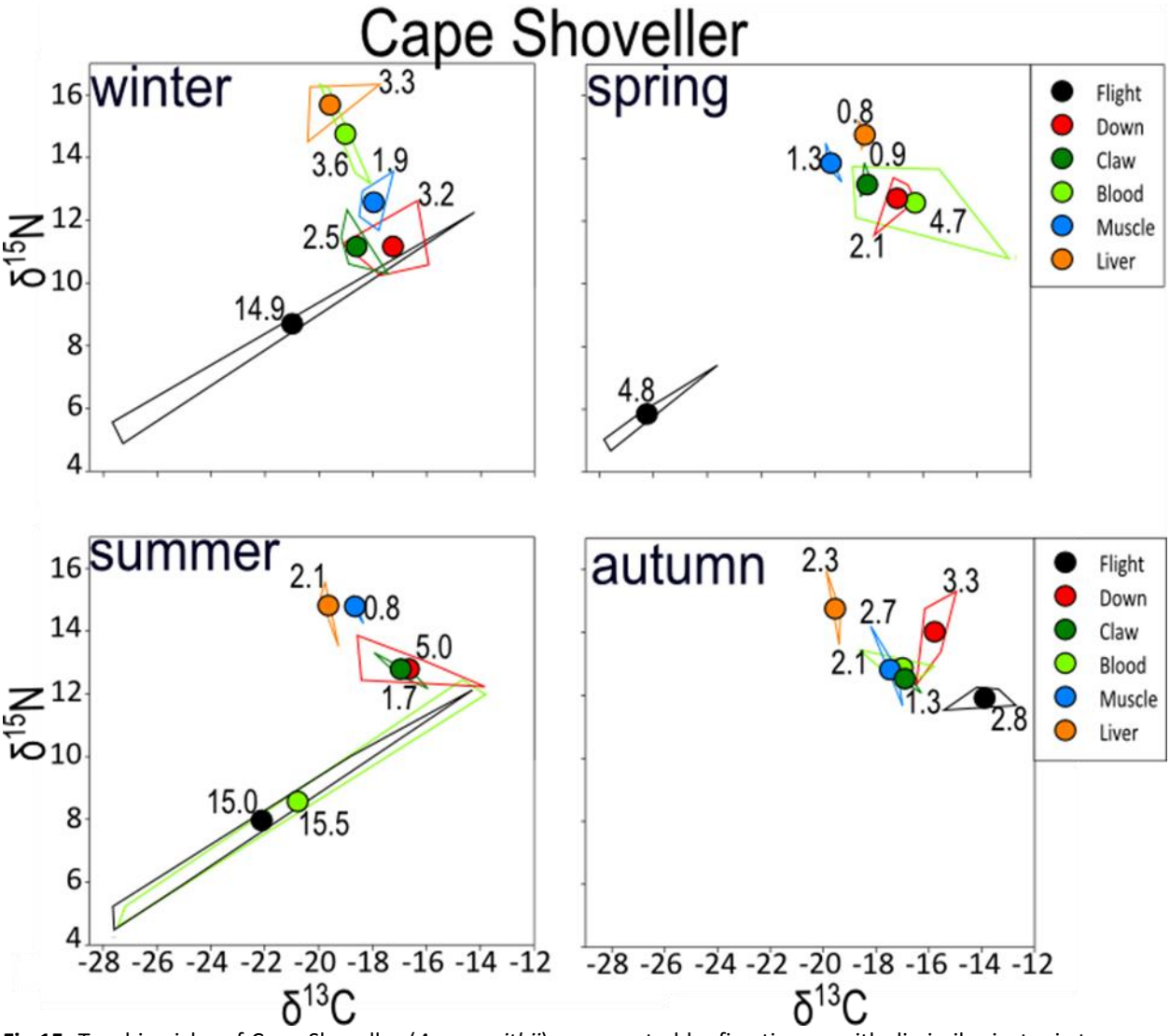

Fig 15: Trophic niche of Cape Shoveller (*Anas smithii*) represented by five tissues with dissimilar isotopic turnover rates. Abbreviations: Flight = primary flight feathers, Down = pectoral down feathers. Solid circles represent the centroid of each tissue type. Numbers next to convex hulls represent the calculated isotopic niche width. Tissues are listed in ascending order according to isotopic turnover rate (i.e. slowest to quickest turnover time; flight feathers > down feathers > claws > blood > muscle > liver).

4.3.2 Cape Teal (*Anas capensis*)

4.3.2.1 Seasonal tissue differences ($\delta^{15}N$ & $\delta^{13}C$)

There was no significant difference between the centroid position of down feathers and claws in winter ($p = 0.456$) and spring ($p = 0.078$), nor a significant difference between the centroid position of blood and muscle during winter ($p = 0.119$) and spring ($p = 0.618$; **Fig 16**). There was however a significant difference in the centroid position of flight feathers compared to down feathers across all seasons ($p < 0.001$). Additionally, there was a significant effect of season on the position of claw and blood centroids ($p < 0.001$ in all seasons; **Fig 16**). The centroid position of down feathers was significantly different to the position of claws during summer and autumn only ($p < 0.001$ in both instances). There was a significant difference between the position of blood and muscle tissue in summer and autumn ($p < 0.001$ in both instances). The position of muscle and liver tissue were not significantly different across all seasons.

4.3.2.2 SIAR models

The diet of Cape Teal was dominated by Mysidacea during winter 2012, as depicted by flight feathers collected in winter and spring 2013 (**Table 8**, **Fig 17**). There was a decrease in the prevalence of micro-invertebrates in the diet of Cape Teal during winter and spring, while the prevalence of Mysidacea in the diet of individuals sampled in both seasons steadily decreased as the seasons progressed from autumn 2013 through to spring 2013 (**Table 8**, **Fig 17**). The diet of Cape Teal during summer 2014 was dominated by marsh plants (44%) and *S. maritima* (42%). Similarly, Cape Teal diet during winter 2013 and summer 2014 was comprised primarily of salt marsh plants (64% and 59%) and *S. maritima* (19% and 24%) (**Table 8, Fig 17**). Cape Teal diet during summer encompassed more crabs, increasing from 10% during spring 2013 to 33% during summer 2014 (**Table 8, Fig 17**). Crabs completely dominated the diet of Cape Teal during autumn 2014 (73%).

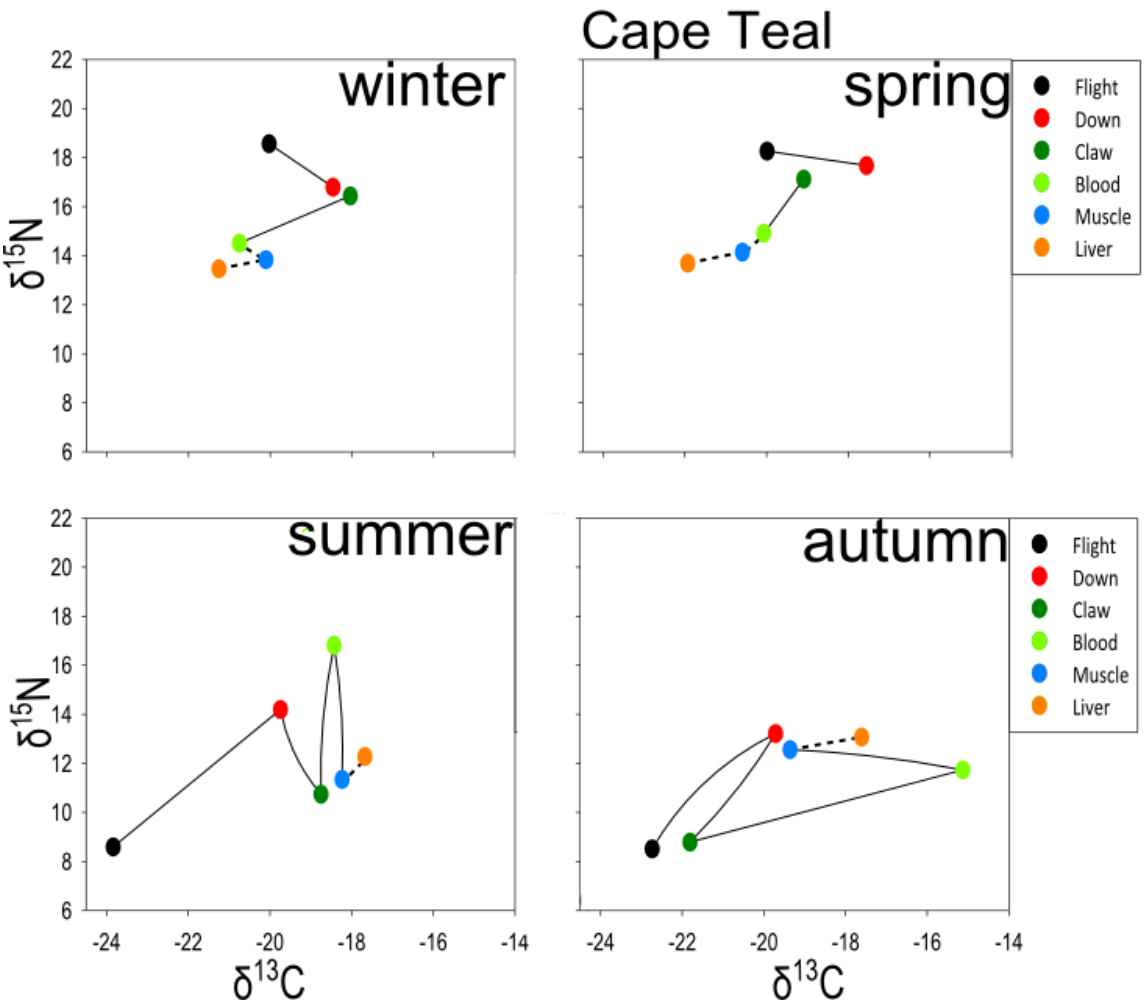

Fig 16: Differences in seasonal tissue centroid positions of Cape Teal (*Anas capensis*). Points represent mean values for tissue type (i.e. centroids).Lines connecting points indicates significant difference in δ-space position. Abbreviations: Flight = primary flight feathers, Down = pectoral down feathers.

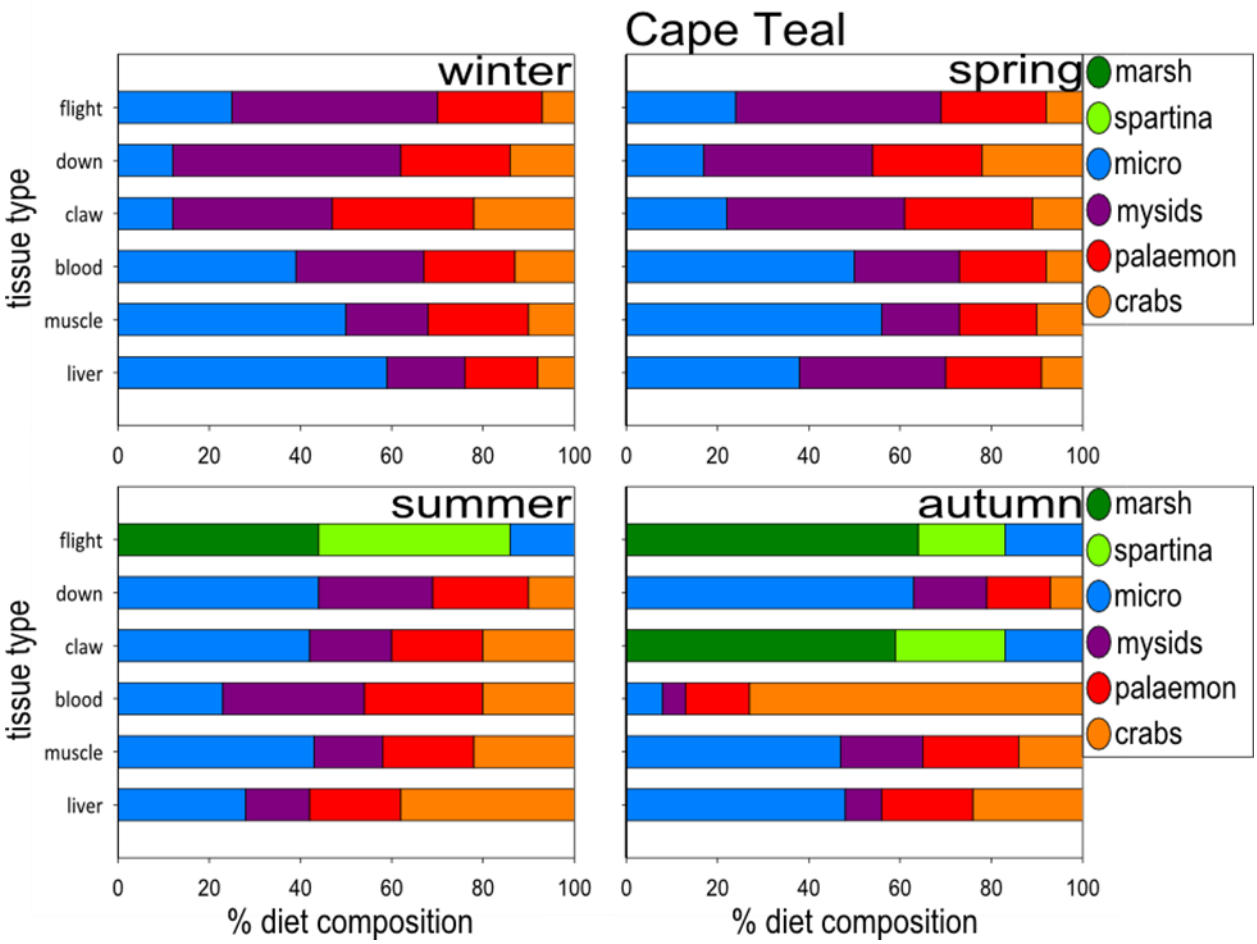

Fig 17: SIAR mixing model output for each tissue type sampled from Cape Teal collected across four seasons. Horizontal bars represent percentage (%) contribution of prey items to overall diet. Abbreviations: marsh = marsh plants, spartina = *S. maritima*, micro = micro-invertebrates, mysids = *Mysidacea*, palaemon = *P. peringueyi*. Tissues are listed in descending order of tissue turn-over rate (i.e. flight > down > claw > blood > muscle > liver).

4.3.2.3 Isotopic niche

Cape Teal fed at a higher trophic level during winter 2012 compared to winter 2013 and spring 2013. The isotopic niche width of Cape Teal during winter and spring increased significantly in size compared to the autumn 2013 and winter 2013 ($p < 0.001$, **Table 9**, **Fig 18**). The isotopic niche width of Cape Teal decreased in size over time ($p < 0.001$, see down feathers and claws vs. flight feathers, **Fig 18**), but increased in size during winter, spring and summer seasons ($p < 0.010$, see blood tissue niche width, **Table 9**). Subsequently, the isotopic niche width as depicted by muscle and liver decreased in a stepwise manner during each season.

The isotopic niche width of Cape Teal remained relatively unchanged from winter 2013 to summer 2014, but increased in size significantly during autumn (see claws, **Table 9**, **Fig 18**). The isotopic niche width as depicted by blood was significantly smaller than the isotopic niche width during summer, as depicted by claws (p = 0.050).

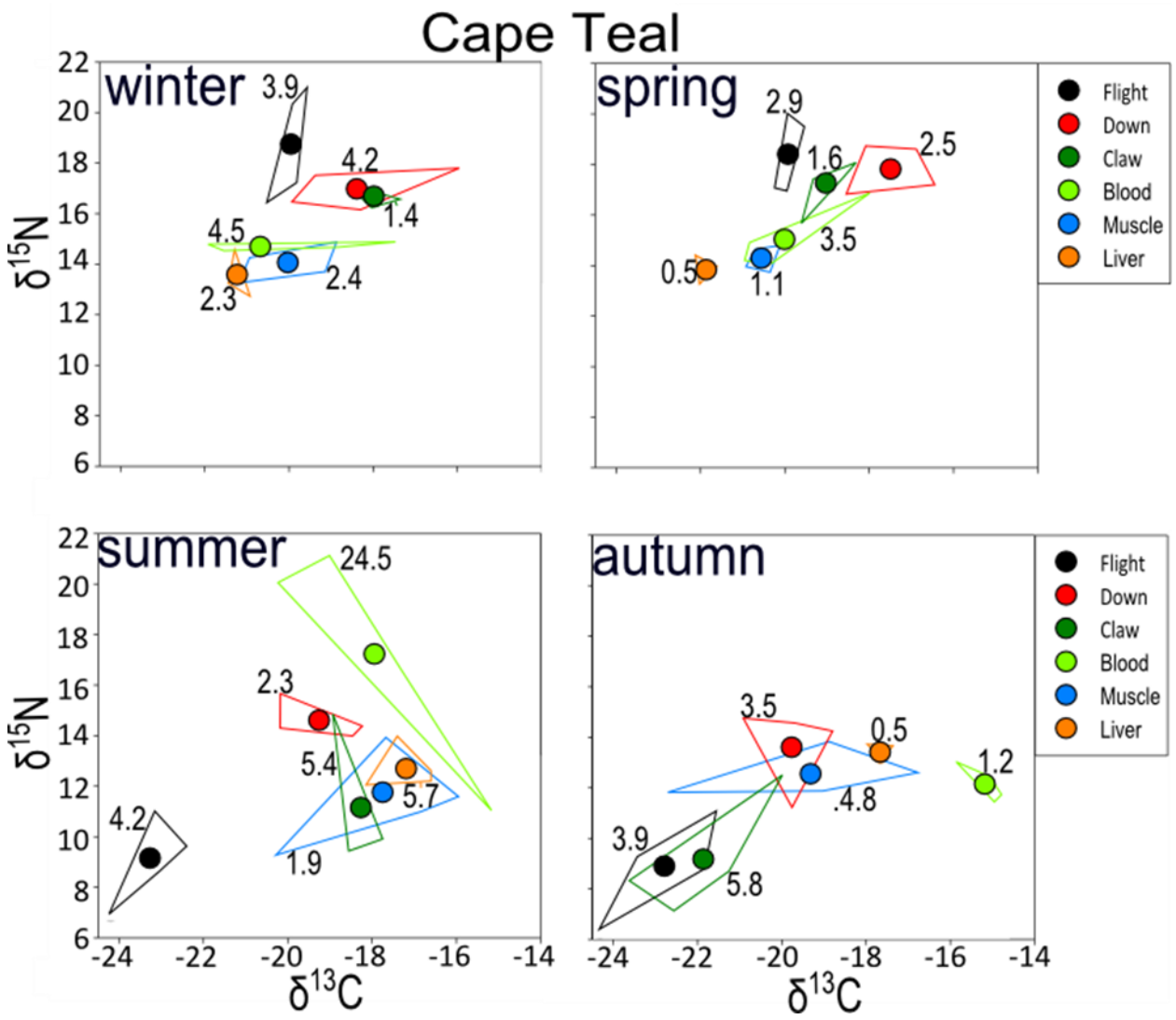

Fig 18: Trophic niche of Cape Teal (*Anas capensis*) represented by five tissues with dissimilar isotopic turnover rates. Abbreviations: Flight = primary flight feathers, Down = pectoral down feathers. Numbers next to convex hulls represent the calculated isotopic niche width. Solid circles represent the centroid of each tissue type. Tissues are listed in ascending order according to isotopic turnover rate (i.e slowest to quickest turnover time; flight feathers > down feathers > claws > blood > muscle > liver).

4.3.3 Yellow-Billed Duck (*Anas undulata*)

4.3.3.1 Seasonal tissue differences

MANOVA revealed that the centroid positions from Yellow-Billed Duck tissues were not significantly different from one another during summer ($F_4 = 2$, $p = 0.098$; **Fig 19**). The position of the down feathers centroid was significantly different from that of flight feathers during winter, spring and autumn ($p < 0.001$ in all instances) (**Fig 19**). Comparably, there were significant differences in the centroid positions of claws and blood ($p < 0.001$ in all instances), and between the centroids of blood and muscle ($p < 0.001$ in all instances) during winter, spring and autumn (**Fig 19**). The centroid position of muscle was significantly different from that of liver during winter only ($p = 0.004$).

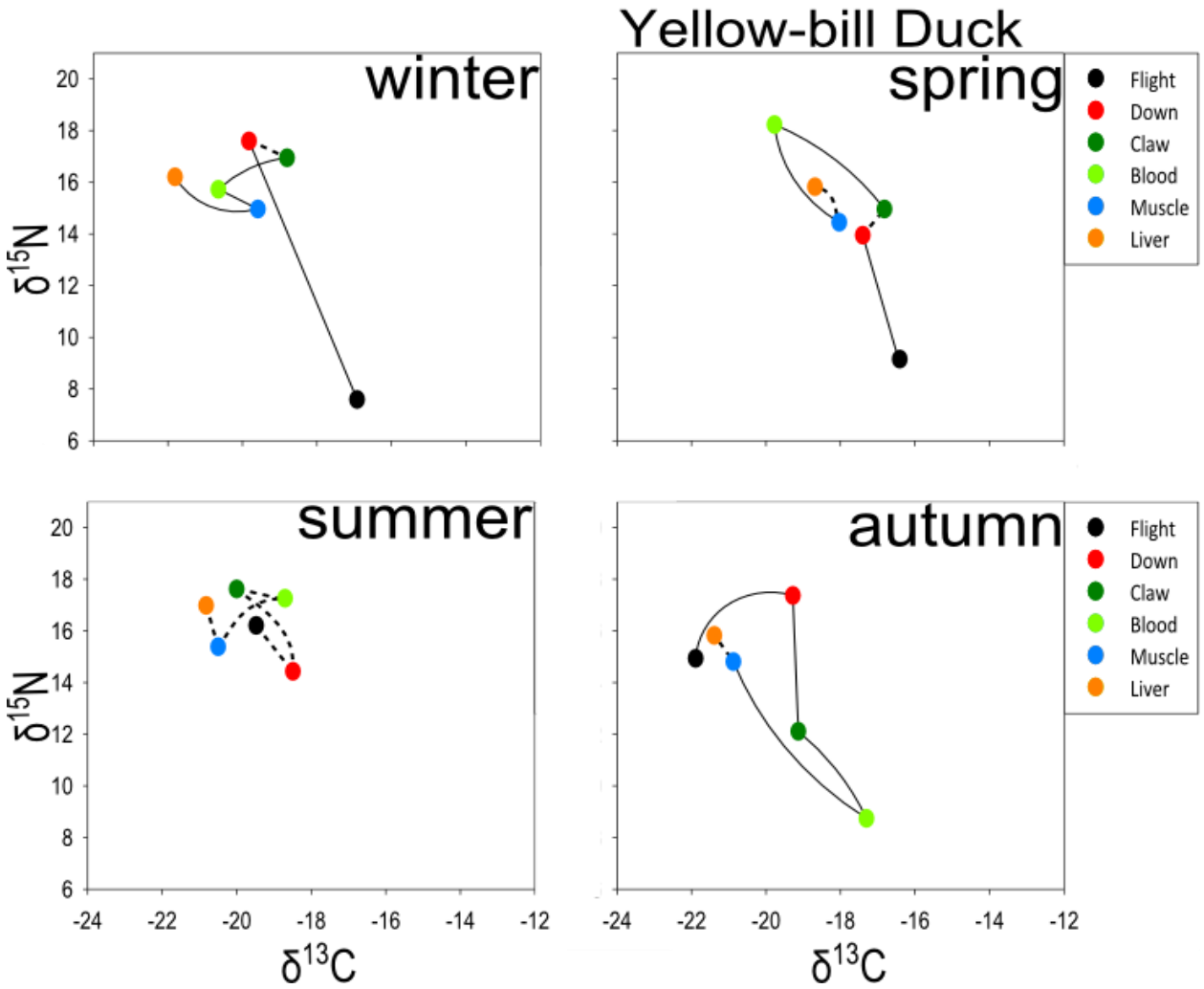

Fig 19: Differences in seasonal tissue centroid positions of Yellow-Billed Duck (*Anas undulata*). Points represent mean values for tissue type (i.e. centroids).Lines connecting points indicates significant difference in δ-space position. Abbreviations: Flight = primary flight feathers, Down = pectoral down feathers.

4.3.3.2 SIAR models

The diet of Yellow-Billed Duck during winter 2012 was dominated by Codium species, as depicted by flight feathers collected in winter and spring 2013 (**Fig 20**). Individuals collected in winter preferentially fed upon Mysidacea during winter, while Yellow-Billed Duck diet during spring encompassed more crabs and *P. peringueyi* compared to the winter diet (**Table 10**, **Fig 20**). Micro-invertebrates and Mysidacea were the preferred dietary resource for summer collected Yellow-Billed Ducks during winter 2013, spring 2013 and summer 2014. The diet of Yellow-Billed Duck incorporated more micro-invertebrates during autumn compared to winter. Similarly, there was an increase in the proportion of Mysidacea in Yellow-Billed Duck diet from early summer (see blood tissue) through to mid-summer 2014 (see muscle and liver tissue, **Fig 20**).

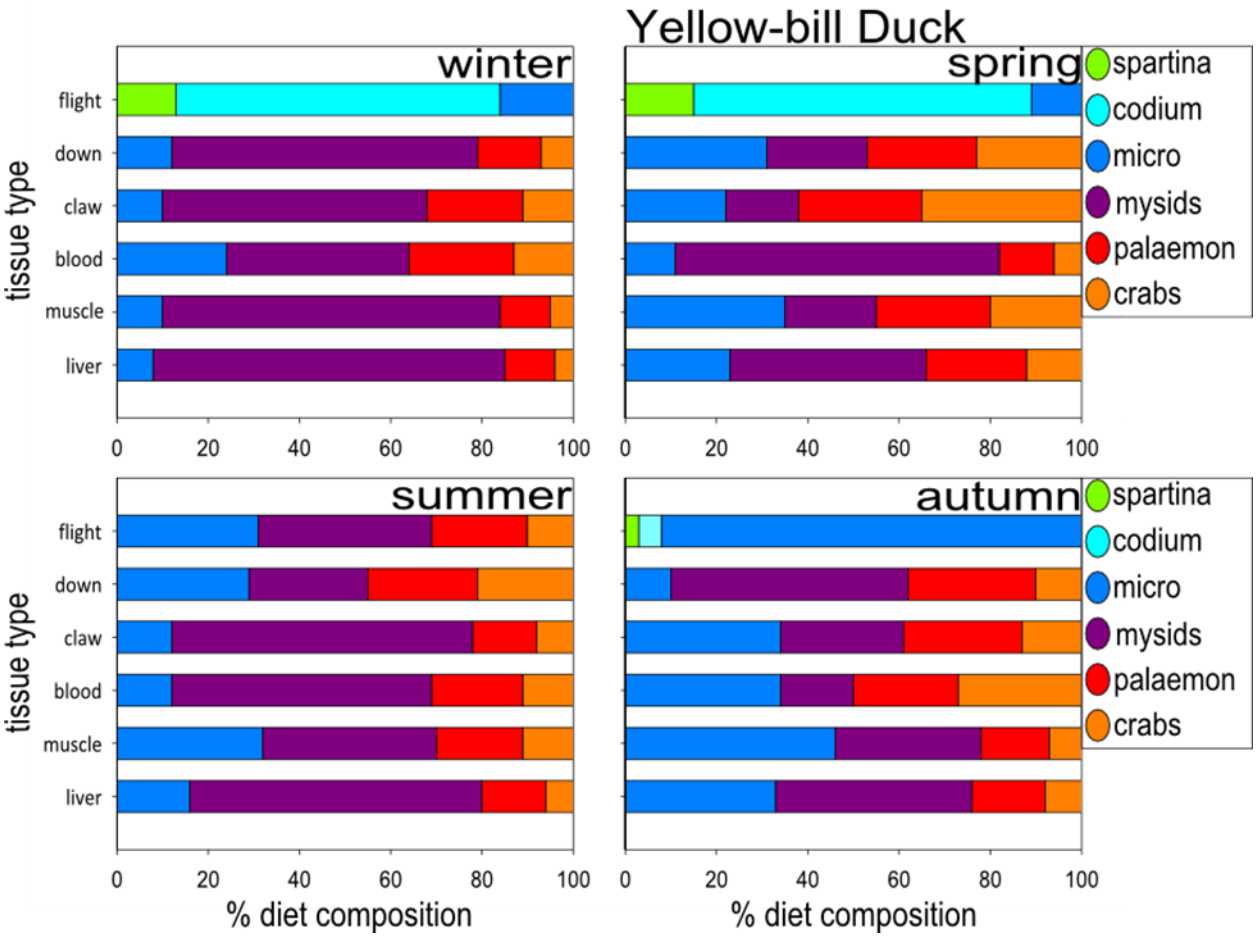

Fig 20: SIAR mixing model output for each tissue type sampled from Yellow-Billed Duck collected across four seasons. Horizontal bars represent percentage (%) contribution of prey items to overall diet. Abbreviations: spartina = *Spartina maritima*, codium = *Codium* spp, micro = micro-invertebrates, mysids = Mysidacea, palaemon = *P. peringueyi*. Tissues are listed in descending order of tissue turn-over rate (i.e. flight > down > claw > blood > muscle > liver).

4.3.3.3 Isotopic niche

The convex hulls depicted by flight feathers did not overlap with the convex hulls of any other tissues of Yellow-Billed Duck collected during winter and spring 2013 (**Fig 21**). The isotopic niche width of Yellow-Billed Ducks collected in winter (F_5 = 3, p = 0.049, see blood tissue) and spring (F_5 = 3, p = 0.038) were largest during winter 2013 (**Table 11**, **Fig 21**). The niche width of Yellow-Billed Duck decreased in size from spring 2013 to summer 2014 (F_5 = 4, p = 0.018). The niche width of Yellow-Billed Ducks collected during autumn was largest during winter 2013 and autumn 2014 (F_5 = 13, p < 0.001). The isotopic niche width of Yellow-Billed Duck decreased in size from winter 2013 to summer 2014 (see flight feathers, down feathers and claws), but expanded significantly during early autumn (see blood). Subsequently, the niche width of Yellow-Billed Duck decreased in size significantly during mid to late autumn (p < 0.001 in both instances, see muscle and liver tissue, **Table 11**, **Fig 21**) compared to early autumn (see blood tissue)

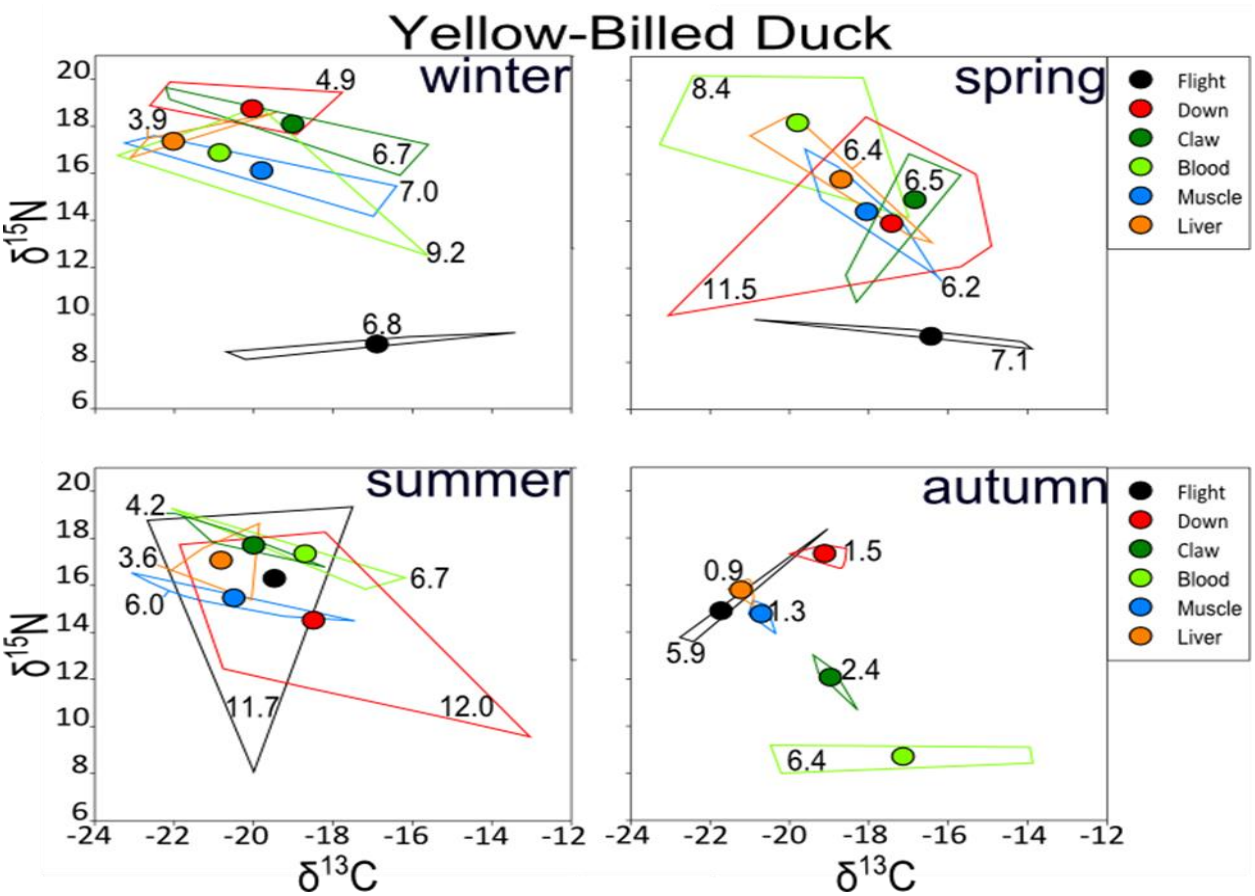

Fig 21: Trophic niche of Yellow-Billed Duck (*Anas undulata*) represented by five tissues with dissimilar isotopic turnover rates. Abbreviations: Flight = primary flight feathers, Down = pectoral down feathers. Numbers next to convex hulls represent the calculated isotopic niche width. Solid circles represent the centroid of each tissue type. Tissues are listed in ascending order according to isotopic turnover rate (i.e. slowest to quickest turnover time; flight feathers > down feathers > claws > blood > muscle > liver).

4.4 Discussion

This study aimed to emphasise the use of stable isotope analysis of several tissues to investigate the feeding ecology of waterbirds over time, I have provided evidence that isotopic signatures can vary greatly amongst tissues within the same individuals, as highlighted by shifts in the centroids of each tissue type per season. The temporal patterns of distribution and dissimilarity between centroids of tissue from each species suggested shifts in diet coincided with resource pulses within the ecosystem over time (MacNeil et al. 2005, Shaner and Macko 2011, see Chapter 3). Cape Shoveller had a plant-based diet during the summer and autumn months, while Cape Teal and Yellow-Billed Duck primarily fed upon aquatic invertebrates over the same time period. The differences in diet amongst the duck species may be attributed to the increased productivity of the ecosystem during summer and early autumn, but may also be an example of resource partitioning mechanisms amongst species to maximise energy intake and minimise direct interspecific competition (Bocher et al. 2014). Consequently, these data support the first hypothesis that the diet of each species varies temporally and with tissue type. Previous studies have recorded similar seasonal diet shifts in pelagic marine birds (e.g. Karnovsky et al. 2008, Martinez et al. 2009, Hedd et al. 2010, França et al. 2011, Wold et al. 2011). By comparison, there is little information pertaining to the seasonal diet shifts of waterbirds feeding in estuaries and the subsequent ramifications of such diet shifts on prey communities.

The temporal niche widths of each duck species provided information on the variety of dietary resources that may be utilised by a group of individuals of a single species. The niche widths of Yellow-Billed Duck during spring and summer were large and inferred wide diet breadth amongst Yellow-Billed Duck individuals. During autumn, however, these same individuals exhibited isotopic signatures that were tightly clustered together, exhibiting a trophic niche that is narrow and implying that Yellow-Billed Ducks fed upon very similar proportions of dietary resources (Martínez del Rio et al. 2009, Araújo et al. 2011). Blood and liver tissues have similar tissue turn-over rate (approximately 5 days difference), but there were significant intra-seasonal differences in the isotopic composition of these tissues in each species. The differences observed in the isotopic composition of tissues with similar turn-over rates (i.e. blood vs. liver) could be attributed to metabolic processes and not necessarily dietary shifts. A caveat that must be considered is isotopic routing amongst tissues. The isotopic composition of tissues is a representation of several biochemical fractions, such as proteins, lipids and carbohydrates, but since lipids and carbohydrates comprise relatively little to no nitrogen content, discrepancies may arise when the diet of consumers is dominated by food sources rich in lipid or carbohydrates (Bearhop et al. 2002).

An unrealistic assumption of mixing models is that nutrients assimilated through digestion are disassembled into their elemental components, which in turn, are reassembled into molecules that constitute tissues (see Wolf et al. 2009 for further explanation). Moreover, the isotopic composition of tissues often reflect the constituent nutrients from whence they were synthesized and not necessarily the bulk diet (Gannes et al. 1997, Bearhop et al. 2002). Additionally, isotopic routing is a mechanism that is known to alter the assimilation of carbon and nitrogen into tissues (Schwarcz 1991). Isotopic routing, if not considered when determining diet through isotopic analysis, can provide inaccurate inferences on diet, particularly when seasonal change in diet are being investigated.

All three duck species may have shifted their foraging activities to focus on specific food items when and where they became available or abundant. Mixing model output showed that the diet of Cape Shoveller fluctuated over time, with significant shifts in diet being observed in each season. The isotopic niche of ducks during autumn was contrary to the expected trend, whereby individuals were expected to have a broad diet breadth because food resources became less abundant in autumn compared to summer. Yellow-Billed Ducks exhibited particularly specialised foraging patterns, indicated by the small isotopic niche widths coupled with SIAR output. Consequently, the second hypothesis that the isotopic niche of each species varies over time is supported. The findings of this study are similar to those of Herrera et al. (2003) and Symes and Woodborne (2009) who revealed resource partitioning and segregation of niche space in forest birds that feed in small fragmented forests. The isotopic niches depicted by Yellow-Billed Duck tissues overlapped more than the isotopic niches of Cape Shoveller and Cape Teal, which suggested that Yellow-Billed Duck utilised a greater breadth of resources available on the mudflats of the Kowie Estuary than the other two species. Presumably, this could be a direct result of changes in resource availability, and the Yellow-Billed Duck population had to expand its diet breadth to maximise energy intake and minimise intraspecific competition for resources (Martínez del Rio et al. 2009). All three duck species sampled in this study exhibited large intraspecific variation amongst the isotopic composition of individuals, suggesting that, while these duck species may be classified as generalist feeders, the population is comprised of specialist feeding individuals (see Bearhop et al. 2004, Araújo et al. 2011 for more detail). $\delta^{15}N$ and $\delta^{13}C$ values from muscle and liver tissue of Cape Shoveller advocated that this species is an isotopic specialists (i.e. tissues with dissimilar turn-over rates have similar isotopic composition, Martínez del Rio et al. 2009), but a dietary generalist (type A generalist; Bearhop et al. (2004). Further investigations that classify and quantify the degree of specialist feeding behaviour amongst duck species along the Kowie Estuary are required to fully support such claims, however.

Several examples of specialist feeding individuals making up a generalist feeding population can be found in the current literature. Vander Zanden et al. (2010) and Thomson et al. (2012) revealed that Loggerhead Turtles displayed evidence of individual feeding specialisation amongst a population of individuals previously considered to be generalist feeders. Likewise, individual specialist foraging behaviour has been observed in several instances in marine-feeding birds (e.g. Bolnick et al. 2002, 2003, Cherel et al, 2007, Jaeger et al. 2009, 2010, Martinez et al. 2009, Catry et al. 2014). The biotic forces driving individual specialisation are numerous and complex (Bearhop et al. 2004, Martínez del Rio, 2009). Nevertheless, studies investigating the causes and consequences of individual feeding specialisation have increased in recent years. Such investigations highlight the complexity of consumer foraging behaviour as well as how individuals and species may partition resources within their environment.

The analysis of the isotopic composition of multiple tissues along with the use of Bayesian mixing models (e.g. SIAR) to reconstruct consumer diets have become invaluable tools for ecologists. However, this study has provided evidence for caveats that need to be taken under consideration. The SIAR models used supported the notion of unique tissue isotopic composition and rapid diet shifts.

The SIAR output for all three duck species showed how tissue choice was an influential factor in diet reconstruction. The mixing model output for tissues of all three species was dissimilar in most instances, with potential prey items contributing unique proportion to the diet per tissue type used. Mixing model outputs are useful when examining the changes in consumer diet over an extended period of time, such as using flight feathers (distant past) versus blood tissue (recent past), the determination of the diet will be solely dependent on the type of tissue used. Analysing the stable isotope signatures of assorted body tissue types with varying turn-over rates from consumers allows ecologists to investigate species foraging patterns and possible shifts in trophic niche at the individual and population level (e.g. Ingram et al. 2009, Martínez del Rio et al. 2009, Vander Zanden et al. 2010, Mihuc and Minshall 1995). The relationship between the isotopic composition of tissues with dissimilar rates of isotopic incorporation can inform the existence of individuals that shift diets over time and of individuals with relatively constant diets (e.g. Quevedo et al. 2009, Vargas et al. 2009). Inferences on the ecology of organisms that are drawn from isotopic composition of tissues rely profoundly on appropriate discrimination factors, and detailed knowledge of tissue turn-over rates (Klaassen et al. 2010, Robbins et al. 2010, Hahn et al. 2012b). Any errors in the discrimination values and turn-over rates, however small, may have significant ramifications for the interpretation of isotopic measurements.

Yet, many isotopic studies on birds rely on a limited number of investigations into discrimination and turn-over times in avian tissues, particularly for tissues other than blood (Pearson et al. 2004., Hobson and Clark 1992a, 1992b, Bearhop et al. 2003, Bauchinger and McWilliams 2009). previous studies have used tissue discrimination factors derived from inter-specific means, or in some instances, even single values measured in other species or tissues (e.g. Robbins et al. 2005, Caut et al. 2009, Hahn et al. 2012). Factors that may influence discrimination values and tissue turn-over rates are tissue type, taxonomic grouping (Caut et al. 2009), temperature (Carleton and del Rio 2005), quantity and quality of dietary protein (McCutchan et al. 2003, Pearson et al. 2003, Williams et al. 2007, but see Dalerum and Angerbjörn 2005) and nitrogen excretion pathways (Vanderklift and Ponsard 2003). However, because the mechanistic foundation that drives the differences in discrimination values and tissue turn-over rates in organisms is not well understood, the strength and direction of these relationships has proven to be unpredictable (Robbins et al. 2010). Consequently, the extrapolation of tissue fractionation values and/or tissue turn-over rates for species in which data are lacking remains problematic (Perga and Grey 2010, Hahn et al. 2012b). As such, there is a need for experimental studies to uncover empirical data on discrimination values and tissue turn-over rates (Martínez del Rio et al. 2009).

Scientists that wish to utilise stable isotopes to unravel foraging habits and reconstruct the diet of consumers, particularly waterbirds, should consider these caveats seriously. Competition for resources amongst individual within and between species is a well-known driver of increased niche variation within populations (Estes et al. 2003, Svanbäck and Bolnick 2007), while the evidence provided from this study of intra-specific stochasticity of individual foraging behaviour could suggest some level of hierarchal structuring within species. Individual isotopic and dietary specialisation may include the use of distinct, spatially separated resources, and could be due to trade-offs in foraging efficiency in relation to habitat use and morphology (e.g. Smith and Skúlason 1996, Svanbäck and Eklöv 2003, Quevedo et al. 2009). Hence, individuals of highly mobile species, such as waterbirds, that may specialise on resources associated with adjacent habitats may offer a new perspective of the role of consumers in food web connectivity and the pressures that they may exert on prey populations, as well as the potential development of stable intrapopulation niche partitioning (Quevedo et al. 2009). Such niche partitioning may limit the efficiency of top consumers to link the fluxes of energy and nutrients across spatially separated food webs, although this has been recognised as an understudied aspect of food web dynamics (Quevedo et al. 2009). To regard natural populations of consumers as homogeneous entities in food webs is misleading, because individual diet specialisation is a ubiquitous trait within the overall population and is present in a large number of taxa (Bolnick et al. 2003).

Furthermore, individual specialisation may be more common in consumers occupying upper trophic levels, because of the increased incidence of strong intraspecific competition (e.g. Bolnick et al. 2003, Herrera et al. 2003, Karnovsky et al. 2008, Quevedo et al. 2009, Martínez del Rio et al. 2009). Consequently, using stable isotopes to distinguish specialist and generalist feeding patterns in consumer populations has increased in popularity in recent years (Vander Zanden et al. 2010) and is a facet of research that ornithologists should consider in future studies. How wild populations of waterbirds utilise all of the resources that are available to them within a defined foraging environment is more complicated than we realise, and how exactly inter- and intra-specific competition may influence the feeding choices of waterbird consumers is a research area that requires our immediate attention, if we are to better understand how these terrestrially-based consumers affect the aquatic food webs in which they feed. Understanding how a species utilises available resources within their environment is tantamount to our understanding of food web interactions and ecosystem functioning.

Fatty acid analysis is not a reliable tool for waterbird diet determination.

5.1 Introduction

Fatty acid (FA) analysis has become a widely used tool by ecologists to study trophic relationships in aquatic food webs (Budge et al. 2006, Iverson 2009, Thiemann et al. 2009). The array of fatty acids present in nature is exceptionally complex, with up to 70 distinct fatty acid signatures being routinely identified within a single organism (Iverson 2008). Numerous studies on birds have demonstrated the transfer of FA through the food web from prey to consumer (e.g. Dalsgaard et al. 2003, Iverson et al. 2004a, Budge et al. 2006). Because of the diverse array of polyunsaturated fatty acids (PUFAs) that originate in phytoplankton, which are invariably transferred up through the food web, the analysis of FAs as dietary tracers have been most comprehensively used in marine based studies (Williams and Buck 2010). Monogastric consumers in marine environments are capable of synthesizing only a small number of fatty acids (Iverson 2008). Therefore, the use of FA profiles to determine marine consumer diets is warranted, as the FA composition of tissues largely reflects diet (e.g. Iverson, 2008; Käkelä et al., 2005; Ramos & González-Solís, 2012; Williams & Buck, 2010).

Fatty acids have three distinct characteristics that make them useful tracer tools of consumer diets and the determination of the overall food web structure (Iverson 2008). Firstly, organisms are able to manipulate FAs through biosynthesis of certain FAs, the modification of carbon chain length, and the ability to introduce double bonds into FAs (Karnovsky et al. 2012). However, consumers that occupy the upper trophic levels of food webs are limited in their biochemical capabilities to manipulate FAs in such processes, which are strongly dependent on phylogenetic classification (Karnovsky et al. 2012). These biochemical limitations increase with increasing phylogenetic order, whereby vertebrates are the most limited in their biochemical manipulation of FAs (Cook 1996). Secondly, unlike most other nutrients, fat is stored in animal bodies in reservoirs where it can later be mobilized to provide fuel for short or long-term energy demands (Iverson 2008). Fatty acids therefore accumulate over time and represent an integration of dietary intake over days, weeks, or months, depending on the organism and its energy intake and storage rates (Käkelä et al. 2010). Thirdly, unlike proteins or carbohydrates that can be broken down to their constituent parts during digestion, FAs are released from ingested lipid molecules during digestion, but are generally not degraded and are taken up by tissues in their basic form (Cook 1996, Käkelä et al. 2005, Karnovsky et al. 2012).

The important consequences of these biochemical restrictions and the uptake of intact FAs by consumers is that individual isomers bioaccumulate through food chains, and they can be traced back to specific origins within the food web (Iverson 2009). It has been hypothesised that wild bird populations select dietary resources that comprise of specific FAs, to allow for maximisation of growth rate, aerobic capacity, absorption efficiency, and enzyme activity (Bairlein and Simons 1995, Crespo and Esteve-Garcia 2001, Bairlein 2002, McWilliams et al. 2002, 2004, Pierce et al. 2004, Pierce and Mcwilliams 2005, Maillet and Weber 2007, Nagahuedi et al. 2009, McCue et al. 2009). Anecdotal evidence has suggested that the FA composition of adipose tissue is consigned directly following absorption of FAs from dietary items (McCue et al. 2009), whereby the determination of FA signatures from adipose tissue can be used as a bioindicator of dietary composition and energy pathways from basal resources in wild birds (e.g. Guglielmo et al. 2002). Birds are capable of considerable structural modification of exogenous FAs prior to their deposition in tissues (Zar 1977, Bairlein and Simons 1995, Bairlein 2002, Egeler et al. 2003, McWilliams et al. 2004), and preferentially channel portomicrons to the liver (Denbow 2000). However, there are insufficient data supporting the notion of FA modifications in individual wild birds (McCue et al. 2009).

In Chapters 3 and 4, the seasonal diet of several waterbirds were examined using $\delta^{15}N$ and $\delta^{13}C$ stable isotope analyses. Based on stable isotope evidence of diet shifts in Chapters 3 and 4, this Chapter aimed to investigate how certain FA may be transported through the aquatic food web to terrestrially-based waterbirds. Through the analysis of the FA profiles of several body tissues sampled from waterbird species along the Kowie Estuary, I posed the following questions: 1) what are the major FA components of waterbird tissues in the Kowie Estuary, 2) is there inter- and intra-seasonal variation in the FA profiles of tissues from species, and 3) can FA analysis provide useful information on the seasonal diet of waterbirds. As such, I tested the following hypotheses: 1) there are significant interspecific differences amongst the FA profile of similar tissues due to variations in diet, as detected by stable isotope data in Chapters 3 and 4, 2) there are significant inter- and intra-seasonal differences amongst the FA profiles of waterbird tissues because of seasonal variations in diets of each species, as shown by stable isotope data, 3) the FA profiles of waterbird tissues reflect the transfer of major FA components from basal resources through to waterbirds, and 4) FA analysis provides accurate information on the seasonal changes in the diets of waterbirds.

5.2 Methods

5.2.1 Sample collection

Adipose, whole blood, muscle and liver tissues were sampled from Cape Shoveller (n = 16), Cape Teal (n = 16), Yellow-Billed Duck (n = 18), Little Egret (n = 16) and Ruff (n = 10) from June 2013- to May 2014 along the Kowie Estuary, Port Alfred, South Africa (Rhodes University ethics permit: ZOOL-09-2012, Department of Environmental Affairs permit: CRO 52/13CR and CRO 53/13CR, see Chapter 2 for site map and Chapter 3 for species descriptions). Tissue samples were placed into ashed (450°C muffle furnace for 4 hours) tin foil envelopes and stored at -80°C for a minimum of 24 h. Frozen tissue samples were freeze-dried at −60°C for 24 h (VirTis BenchTop 2K), and ground into fine powder using individually lipid-cleaned pestle and mortars. Thirty to sixty milligrams of each dried tissue sample was weighed into 15ml glass tubes. Lipids were extracted from blood using the Indarti et al. (2005) method, while the neutral lipid fraction was extracted from adipose, muscle and liver tissue. Neutral lipids are the major components of energy rich fat depots and therefore more accurately reflect diet intake, compared to polar lipids which are major components of cell and organelle membranes (e.g. Karasov and Martinez del Rio 2007). There are several caveats surrounding FA analysis of blood collected from wild birds (see Williams and Buck 2010 for details). As such, the Indarti et al (2005) method was used for whole blood because FAs in blood are found principally in membrane phospholipids.

5.2.2 Lipid extraction

5.2.2.1 One-Step extraction

Weighed homogenised samples were placed into 15 ml screw cap test tubes with 2 ml of 0.01% BHT infused chloroform ($CHCl_3$). Test tubes were then flushed with N_2, sealed with teflon tape and vortexed for 10 s. These samples were placed into a 100°C oven for 30 min. Tubes were allowed to cool to room temperature, before 1 ml of MilliQ water was added to each sample and mixed with a vortex. Samples were placed into a centrifuge at 3000 rpm for 3 min. The upper aqueous layer was discarded, and the remaining sample was dried with sodium sulphate (Na_2SO_4). Samples were rinsed through cotton-wool plugged pipettes and evaporated under a gentle N_2 stream. When samples were reduced to less than 1 ml, they were transferred to 2 ml vials, whereby they were evaporated until dryness.

Before sealing with N_2 and Teflon tape, 0.5 ml of hexane was added to each sample. Samples were stored at -20°C until required for chromatography.

5.2.2.2 Neutral Lipid extraction

Similarly to the Indarti et al. (2005) method, all samples were placed into 15 ml screw-top test tubes. Subsequently, 1 ml of ice-cold methanol (MeOH) was added to each test tube, the sample was vortexed, and then placed into a sonicator with ice for 4 min. After sonication, samples were vortexed, flushed with N_2, sealed and stored at -20°C for 24 h. Samples were filtered through cotton wool plugged pipettes that were pre-rinsed with a 2:1 chloroform: methanol ($CHCl_3$: MeOH) solution. A 1.5 ml aliquot of 0.9% potassium chloride (KCL) was added, the sample vortexed, centrifuged at 3000 rpm for 3 min, and the top aqueous layer was removed and discarded. To each test tube, a further 0.5 ml KCL and 0.5 ml MeOH was added, vortexed and centrifuged as done so previously. The top aqueous layer was removed and the remaining sample was dried with sodium sulphate (Na_2SO_4). The sample was filtered through a Na_2SO_4 topped, cotton-wool plugged pipette, and evaporated until dryness under an N_2 stream. A 0.5 ml aliquot of $CHCl_3$ was further added to the dried sample, sealed and stored at -20°C until required for chromatography. To extract neutral lipids from the total lipid extract, glass pipettes were plugged with glass-wool and placed into a muffle furnace at 450°C for 4 h. Approximately 0.8 g of silica gel was placed into each glass-wool ashed pipette. The silica gel was activated by placing the pipettes into a 100°C oven for 1 h. Once removed from the oven pipettes were allowed to cool for approximaly 20 min. With the pipette held upright in a retort stand, 6 ml of dried MeOH was eluted through the silica column into a waste beaker. As the last of the MeOH reached the surface of the silica, 6 ml of Na_2SO_4 dried $CHCl_3$ was eluted, ensuring that the surface of the silica gel was not disturbed. When the last of the $CHCl_3$ had been eluted through the column, 6ml of 98:1:0.5, ($CHCl_3$: MeOH: formic acid), was eluted. When half of the solvent had passed through the column, the waste beaker was replaced by a lipid cleaned 15 ml vial. The sample extract was added to the column when all of the 98:1:0.5 solvent had been eluted. The sample vial was rinsed with $CHCl_3$, and the rinse was eluted through the silica. Finally, 8 ml of 98:1:0.5 solvent was eluted through the column. The sample extract and solvent mixture in the 15 ml collection vial was evaporated under an N_2 stream to as small a volume as possible without drying, then 1.5 ml of dried methylene chloride (DCM) was added. The sample vial was sealed and stored at -20°C until required.

5.2.3 Fatty acid methyl ester (FAME) synthesis

All sample vials containing extracted FAME were topped with hexane, and injected into an Agilent 7890 gas chromatograph (GC) fitted with a ZB-WAXplus 320 column and a flame ionization detector. Helium was the carrier gas, and 1 µl of each FAME sample was manually injected at 260 °C (inlet temperature) with the oven set at 150 °C. After 5 min, the oven temperature was raised to 225 °C at 2.5 °C/min. FAME peaks were visualized using ChemStation™ chromatography software, identified by comparison with retention times using external standards (marine PUFA no. 1, 37 component FAMEs mix; Supelco). Peak identification was confirmed using mass spectrometry (MS) on an Agilent 7000A GC/MS-QQQ coupled with a NIST 08 MS library, with column and methods identical to the GC runs. Each FA was measured as a proportion of the total FAs (%TFA). FA names are abbreviated as Ca:bω*x*, where, a is the number of carbon atoms, b is the number of double bonds, and *x* is the position of the first double bond from the methyl end of the molecule (Budge et al. 2006).

5.2.4 Data analysis

Analysis of similarity (ANOSIM) was used to determine significant intra and inter-seasonal differences in the FA profiles of waterbird tissues. Similarly, ANOSIM was used to determine interspecific differences amongst similar tissues. Similarity percentages (SIMPERs) were used to identify influential FAs. Additionally, non-metric dimensional scaling (nMDS) based on Euclidean distance matrices was used (Kruskal and Wish 1978). I compared SIMPER results with the loading results from principal components analysis (PCA) using data as outlined above. I performed this SIMPER-PCA comparison as a visual aid only, and the PCA loadings served as guideline estimates to superimpose the SIMPER results onto the nMDS plots. Furthermore, I tested for significant intraspecific differences in the proportions of saturated FAs (SFAs), monounsaturated FAs (MUFAs), polyunsaturated FAs (PUFAs) amongst tissues using one-way ANOVA (95 % significance level) and Tukey's post-hoc test at 5 % significance. The dominant FAs of waterbird tissues were compared with major FAs detected in basal resources and major foods sources (extrapolated from Bergamino and Richoux 2014). All statistical analyses were performed in PAST 3.01 (Hammer et al. 2001).

5.3 Results

5.3.1 Interspecific and inter-seasonal differences

ANOSIM revealed significant interspecific differences between the FA profiles of adipose, liver, muscle and blood during each season ($p < 0.001$ in each season). SIMPER revealed that during winter 2013, the SFA C16:0 was principally responsible for the interspecific differences observed amongst the FA profiles of adipose (25%), liver (52%), muscle (43%) and blood (45%). During spring 2013, SFAs C16:0, C17:0 and C18:0 were responsible for the major interspecific differences observed amongst adipose (37%) and muscle (43%) but PUFAs C22:5ω3, C22:6ω3 and C20:5ω3 were responsible for the major differences observed amongst liver (39%) and blood (50%). Converse to tissues collected during winter, PUFAs C20:5ω3, C18:2ω6, C22:5ω3 and C22:6ω3 were responsible for the major interspecific differences observed amongst all tissues during summer 2014. Similarly, PUFAs (primarily C20:5ω3) were responsible for the major interspecific differences observed amongst the FA profiles of adipose (56%) and blood (44%). SFAs (51%) and MUFAs (40%) were responsible for the major interspecific differences detected amongst liver and muscle respectively.

ANOSIM revealed significant inter-seasonal differences amongst the FA profiles of each tissue collected from each species. The FA profile of adipose, blood, muscle and liver collected from Cape Shoveller, Cape Teal and Yellow-Billed Duck and Little Egret were significantly different every season ($p < 0.05$ in each instance). PUFAs were responsible for the major inter-seasonal differences detected amongst Yellow-Billed Duck tissues (39% - 63%) and Little Egret (31% - 58%). Similarly, PUFAs accounted for the major differences observed amongst adipose tissue collected from Cape Shoveller (59%) and Cape Teal (69%). SFAs were responsible for the major inter-seasonal differences observed amongst the FA profiles of blood, muscle and liver collected from Cape Teal (43% - 52%), while SFAs accounted for 46% of the observable inter-seasonal differences detected amongst Cape Shoveller muscle. The FA profile of adipose collected from Ruff during spring was significantly different to adipose collected during summer ($p = 0.031$), where the SFA C16:0 (55%) and MUFA C16:17 (33%) accounted for the major differences observed between the seasons. There were no significant inter-seasonal differences detected amongst the fatty profiles of blood ($p = 0.175$), muscle ($p = 0.085$) and liver ($p = 0.195$) collected from Ruff.

5.3.2 Intraspecific differences

5.3.2.1 Cape Shoveller

There were significant differences detected in the FA profile of tissues collected from Cape Shoveller each season. Major FAs detected in the tissues of Cape Shoveller included SFAs C16:0 and C18:0, MUFAs C16:1ω7 and C18:1ω9, and PUFA C20:5ω3 (**Table 12**). More specifically, C16:0 constituted a high proportion (15% - 46%) of the FA profile of Cape Shoveller tissues during winter, spring and autumn. Meanwhile, PUFA C20:5ω3 was the major FA component of Cape Shoveller tissues during summer (19% - 41%). Adipose was dominated by PUFAs during spring (55% ± 4%, $F = 91$, $p < 0.001$), summer (65% ± 7%, $F = 122$, $p < 0.001$) and autumn (49% ± 5%, $F = 91$, $p < 0.001$), while PUFAs contributed the least to the overall FA profile of adipose during winter 2013 (12.4% ± 3%, $F = 46$, $p < 0.001$) (**Fig 21**). The proportion of PUFAs in the FA profile of adipose and muscle peaked during summer, with an increase in PUFAs from winter to summer, but with a subsequent decrease in the proportion of PUFAs from summer to autumn (**Fig 21**). There was no clear trend in the abundance of SFAs, MUFAs or PUFAs in the FA profiles of blood and liver (**Fig 21**). ANOSIM revealed significant intra-seasonal differences amongst tissues ($p < 0.001$ in each season).

The SFAs C16:0, C18:0, MUFAs C16:1ω7, C18:1ω9, C18:1ω7 and PUFA C20:5ω3 were responsible for more than 60% of the dissimilarity of FA profiles observed amongst tissues. The convex hulls of Cape Shoveller tissues did not exhibit any overlap in any season (see **Fig 21**). Significant inter-seasonal differences were detected for each tissue type ($p < 0.001$ in all instances). Despite the FA profiles of each tissue collected being significantly different from one another each season, Cape Shoveller tissues were generally dominated by the SFA C16:0 and PUFA C20:5ω3 (**Table 12**). During winter, adipose, muscle and liver were similarly dominated by C16:1ω7 (11% - 21%), while blood contained high proportions of C18:3ω4 (12.4% ± 3.9%) and C18:3ω3 (11.3% ± 3.8%). Cape Shoveller tissues were dominated by the PUFA C20:5ω3 during summer (19% - 41%), with C16:0 comprised a large percentage of the FA profile of blood (17.9% ± 6.5%), muscle (19% ± 5.9%) and liver (23% ± 3.8%). Blood collected during autumn 2014 was similar to winter 2013, whereby PUFAs C18:3ω3 (10.7% ± 3.5%) and C18:4ω3 (10% ± 4%) were the major FA components.

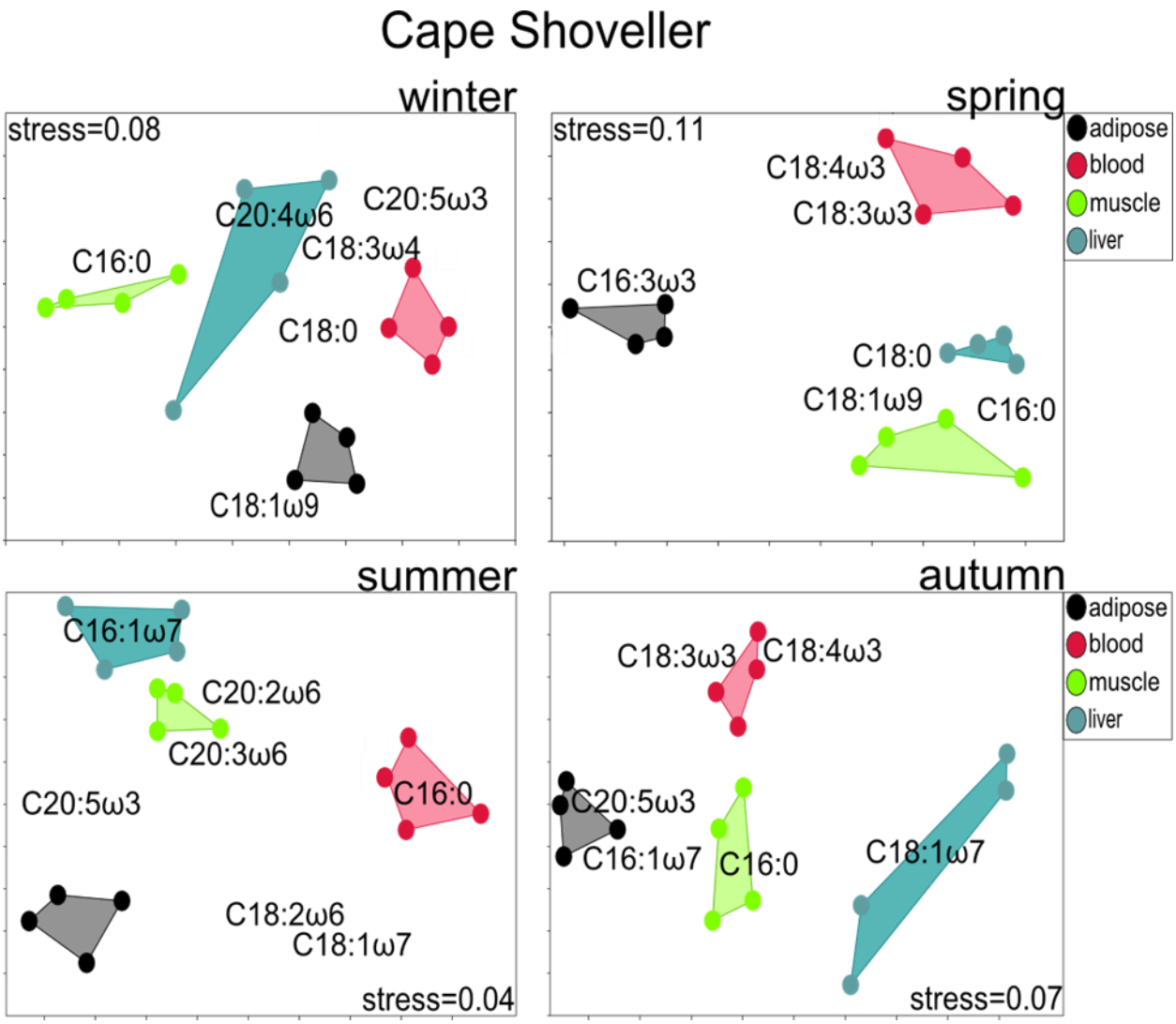

Fig 22: Non-metric multidimensional scaling output using the FA profiles of adipose, blood tissue, muscle and liver collected from Cape Shoveller across four seasons (winter 2013 to autumn 2014). Influential FAs that separated the four tissue types (derived from SIMPER and PCA) are superimposed in each seasonal plot.

5.3.2.2 Cape Teal

Cape Teal exhibited an increase in PUFAs in the FA composition of adipose tissue, blood tissue and muscle tissue from winter to summer, but a decrease in abundance of PUFAs during autumn (**Fig 22, Table 13**). Liver tissue was unique in that the proportion of PUFAs peaked during spring but then decreased from spring to autumn (**Fig 22**). Cape Teal tissues demonstrated similar patterns of MUFA abundance across the seasons. The proportion of MUFAs in tissues peaked during winter 2013, then decreased to its lowest proportion during summer but then increased in proportion from summer to autumn (**Fig 22**). SFAs contributed the smallest proportion to the FA profiles of Cape Teal tissues, despite C16:0 being a major FA component in all tissues (**Fig 22**). Significant intra-seasonal differences were detected amongst the FA profiles of tissues during spring ($p < 0.001$), summer ($p < 0.001$), and autumn ($p < 0.001$). ANOSIM revealed that adipose was significantly different from blood ($p = 0.027$), muscle ($p = 0.029$) and liver ($p = 0.031$) during winter, but there were no significant differences detected amongst blood, muscle and liver during winter. C16:0 was a primary FA component of blood (31.2% ± 2%), muscle (47% ± 7%) and liver (52% ± 16%) during winter (**Table 13**), but adipose was dominated by C18:1ω9 (16% ± 2%) and C22:5ω3 (14% ± 1%). SFAs C16:0, C17:0, MUFAs C18:1ω9, C18:1ω7, and PUFA C20:5ω3 were primarily responsible for the dissimilarities observed amongst the FA profiles of Cape Teal tissues. There was a shift in the prevalence of specific FAs in each tissue as the seasons progressed from winter to summer. There was a decrease in C16:0 but an increase in C18:1ω9 and C20:5ω3 in the FA profile of blood, muscle and liver from winter 2013 to summer 2014. The MUFA C18:1ω9 increased in prevalence in blood (Δ17%) and muscle (Δ20%) during spring. C20:5ω3 was the dominant FA component in blood (26%), muscle (15%) and liver (29%) during summer. Similarly, PUFAs C22:5ω3 and C22:6ω3 were the dominant FAs in Cape Teal adipose during summer.

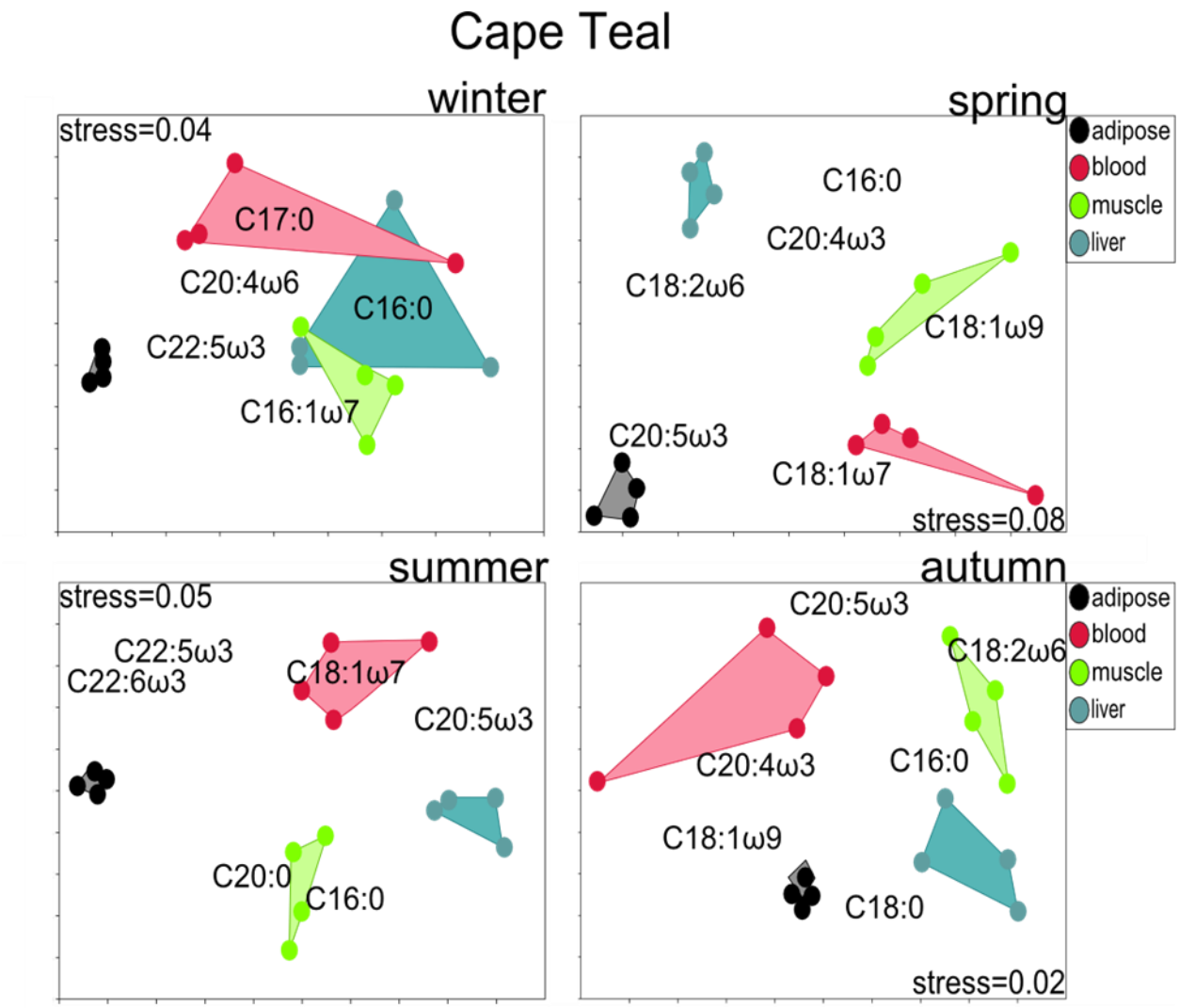

Fig 23: Non-metric multidimensional scaling output using the FA profiles of adipose, blood, muscle and liver collected from Cape Teal across four seasons (winter 2013 to autumn 2014). Influential FAs that separated the four tissue types (derived from SIMPER and PCA) are superimposed in each seasonal plot.

5.3.2.3 Yellow-Billed Duck

There was an increase in the proportion of PUFAs in the FA profiles of adipose of Yellow-Billed Duck from winter 2013 to autumn 2014 (**Fig 27**). Similarly, PUFA's dominated the FA profile of blood during spring (69 % ± 9%, $F = 108$, $p < 0.001$) and summer (61% ± 5%, $F = 205$, $p < 0.001$). PUFAs in muscle demonstrated the inverse pattern to blood whereby the proportion of PUFAs peaked during winter 2013 (60% ± 4%) and was lowest during summer 2014 (36% ± 6%, **Fig 25, Table 14**). Meanwhile, MUFAs in blood peaked during summer 2014 (**Fig 25**). Muscle was significantly depleted in SFAs across all seasons (**Fig 25**). Yellow-Billed Duck liver demonstrated no clear inter-seasonal trends in the abundance of SFAs, MUFAs and PUFAs. ANOSIM revealed significant intra-seasonal differences amongst the FA profiles of Yellow-Billed Duck tissues ($p < 0.001$ in each season; **Fig 25**). There was minimal overlap amongst tissues during the seasons. There was a small amount of overlap between muscle and liver during winter, as well as overlap between adipose and liver during spring (see **Fig 28**). Dominant FAs detected in all tissues included SFAs C16:0, C18:0, MUFAs C18:1ω9, C18:1ω7, and PUFAs C18:2ω6, C20:5ω3, C22:5ω3 and C22:6ω3 (**Table 14**). PUFA isomers of C18 and C20 were primarily responsible for the dissimilarity amongst the FA profiles of tissues sampled from Yellow-Billed Duck during winter, spring and summer, while C18:1ω9 and C18:1ω7 alone were responsible for 49% of the dissimilarities observed amongst tissues during autumn (**Fig 25**).

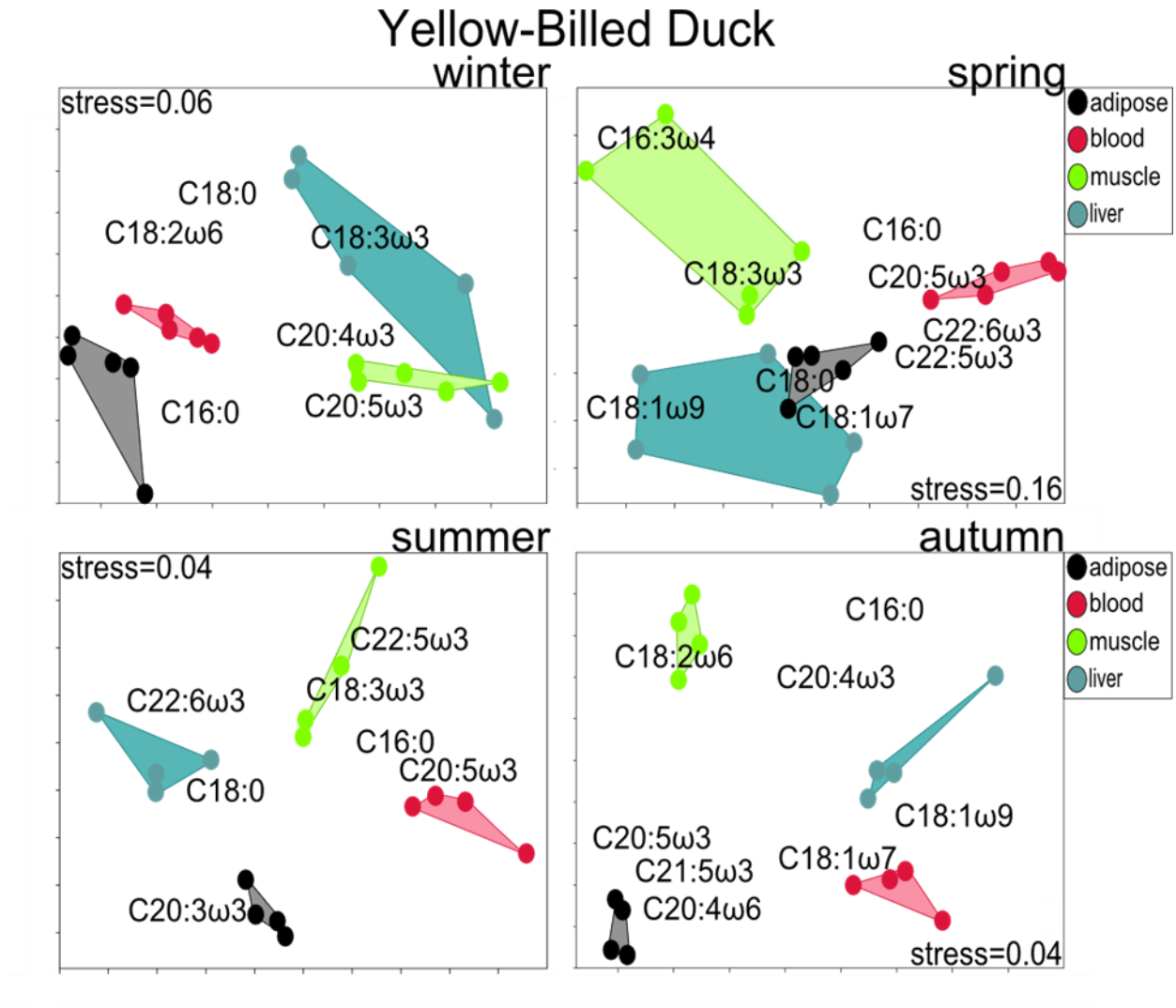

Fig 24: Non-metric multidimensional scaling output using the FA profiles of adipose tissue, blood, muscle and liver collected from Yellow-Billed Duck across four seasons (winter 2013 to autumn 2014). Influential FAs that separated the four tissue types (derived from SIMPER and PCA) are superimposed in each seasonal plot.

5.3.2.4 Little Egret

PUFAs dominated the FA profiles of blood and muscle collected from Little Egret, with C20:5ω3 being particularly prominent (**Table 15**). ANOSIM revealed significant differences in the FA profiles of each tissue during each season ($p < 0.001$ in each instance). SIMPER detecting PUFAs C18:2ω6, C18:3ω4, C20:4ω6, 20:5ω3 and C22:5ω3 as being responsible for more than 45% of the variability observed amongst tissues. Adipose and liver exhibited increases in the proportions of PUFAs from winter to autumn. Blood (49% - 53%) and muscle (47% - 60%) did not display a significant changes in the proportion of PUFAs winter to autumn. Generally, MUFAs comprised the lowest proportions of all tissue FA profiles across the study period (< 15%). Blood (25% ± 2% - 32% ± 4%, $p = 0.047$), muscle (34% ± 4% - 45% ± 9%, $p = 0.001$) and liver (29% ± 3% - 41% ± 5%, $p = 0.050$) did not exhibit a significant change in the proportion of SFAs in their FA profiles from winter to autumn. There were no significant difference in the proportion of SFAs and PUFAs in the FA profiles of Little Egret tissues throughout the seasons, while MUFAs constituted significantly the lowest proportion of Little Egret tissues.

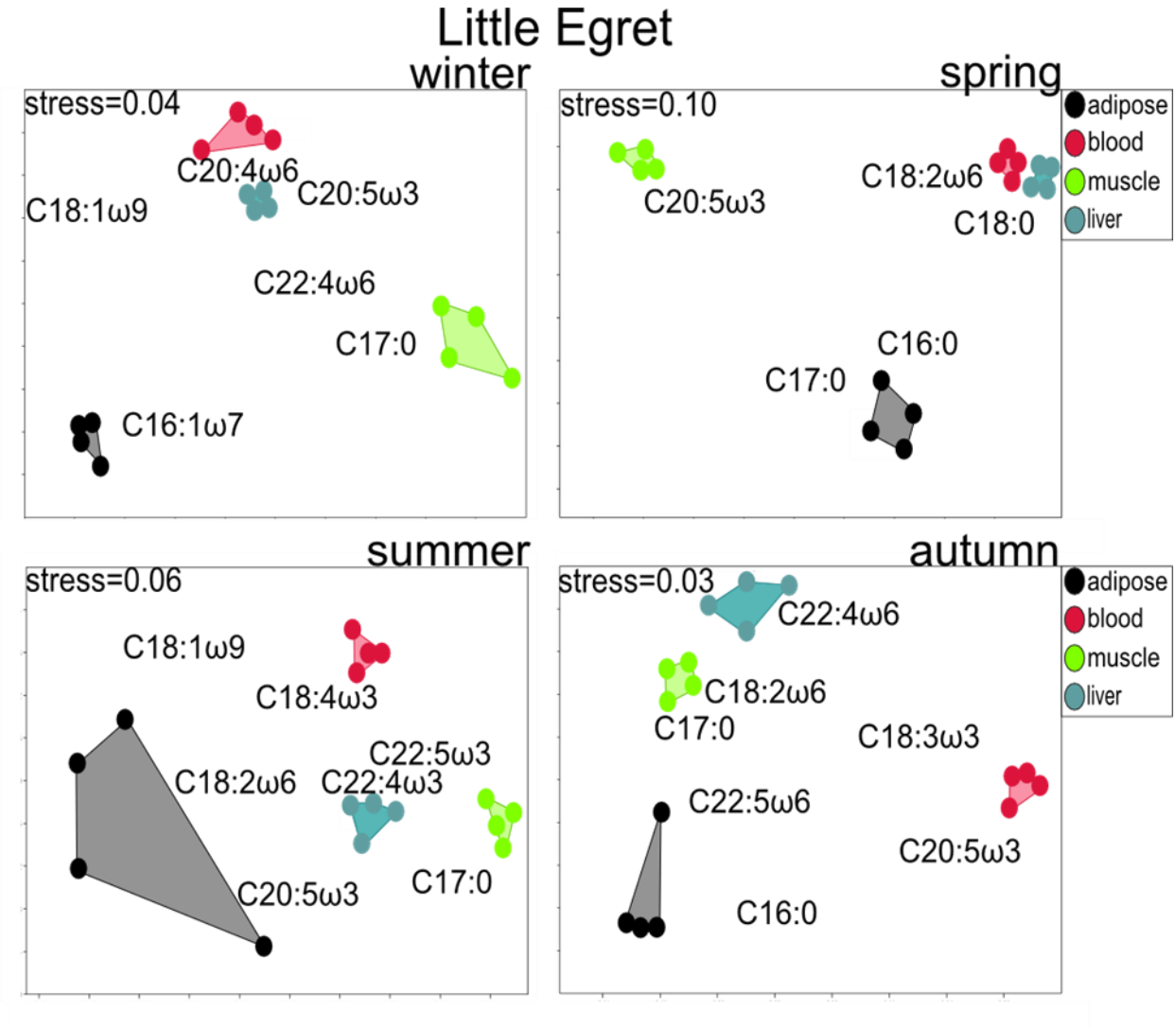

Fig 25: Non-metric multidimensional scaling output using the FA profiles of adipose, blood, muscle and liver collected from Little Egret across four seasons (winter 2013 to autumn 2014). Influential FAs that separated the four tissue types (derived from SIMPER and PCA) are superimposed in each seasonal plot.

5.3.2.5 Ruff

PUFAs dominated the FA profiles of Ruff blood (55% ± 13%) and liver (48% ± 12%) during spring, while SFAs dominated muscle during spring (51% ± 8%). There were no significant differences detected between the proportions SFA, MUFAs and PUFAs in adipose during spring (F= 3, p = 0.828). SFAs significantly dominated the FA profiles of adipose tissue (F = 28, p = 0.021) and liver (f = 14, p = 0.034) during summer. Conversely, PUFAs comprised the greatest proportion of the muscle FA profile during summer, while there were no significant differences detected in the proportions of SFAs (39% ± 18%) and PUFAs (44% ± 19%) in blood during summer (F = 4, p = 0.713). During the spring and summer, the FA profiles of Ruff tissues were dominated by the SFA C16:0 (16% - 35%), MUFA C18:1ω9 (6% - 13%) and PUFA C20:5ω3 (9% – 27%, **Table 16**). SIMPER revealed that SFAs (39%) and PUFAs (42%) were responsible for the majority of the dissimilarities observed amongst tissues during spring and summer. The SFA C16:0 was a major FA component in all tissues in during spring (35%) and summer (33%; **Table 16**). ANOSIM revealed significant differences amongst the FA profiles of Ruff tissues during spring and summer (p = 0.001 in both instances). Despite the significant differences in the FA composition of tissues, there was a large amount of overlap between the convex hulls of blood and liver during spring (**Fig 25**). Conversely, there was no overlap amongst the convex hulls of all other tissue during spring, and no overlap amongst tissues during summer.

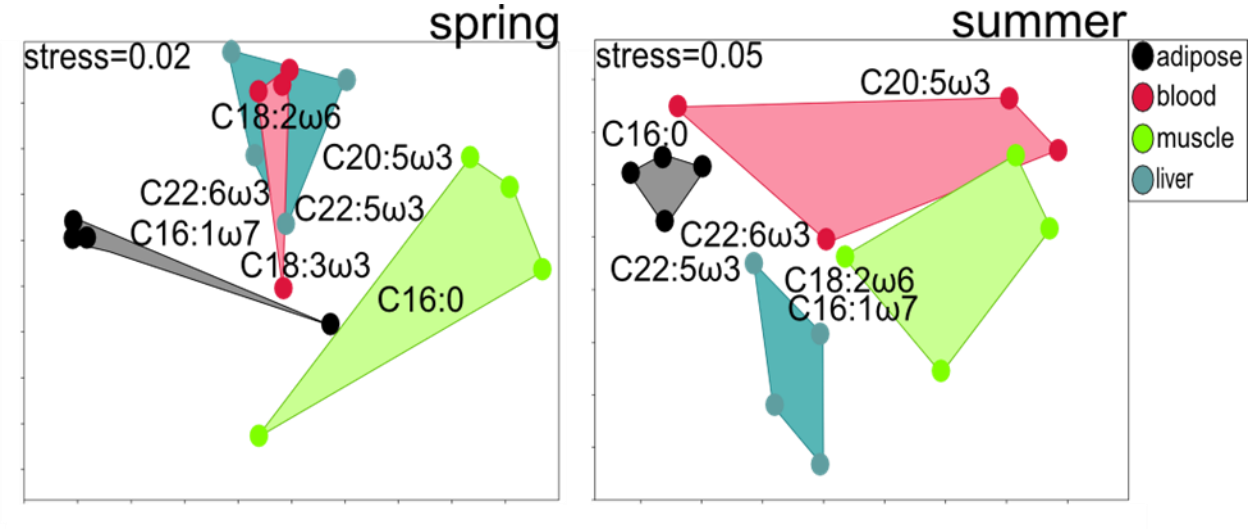

Fig 26: Non-metric multidimensional scaling output using the FA profiles of adipose, blood, muscle and liver collected from Ruff during spring 2013 and summer 2014 only. Influential FAs that separated the four tissue types (derived from SIMPER and PCA) are superimposed in each seasonal plot.

5.4 Discussion

The results of the FA analysis of waterbird tissues lead to similar conclusions to those drawn from stable isotope data in Chapters 3 and 4. Chapters 3 and 4 provided evidence for inter- and intra-seasonal diet variation in waterbirds. FA analysis of waterbird tissues similarly provided support for these findings, whereby significant inter- and intra-seasonal differences were detected in the FA profiles of waterbird tissues, particularly in the tissues of all three duck species and Ruff (see **Fig 22** – **26**). Despite some tissues having similar turn-over rates, such as muscle and liver, significant inter-seasonal differences were detected amongst the FA profiles of these tissues. Similarly, the significant interspecific differences detected amongst the FA profiles of similar tissues further supports the inter- and intra-seasonal diet variation findings of Chapters 3 and 4. Consequently, I support the first and second hypotheses that there are interspecific differences amongst the FA profiles of similar tissues and inter- and intra-seasonal differences in the FA profiles of waterbird tissues.

This study also provides evidence that certain FAs are transferred through the Kowie Estuary food web relatively unchanged, from basal resources (detritus, particulate organic matter (POM) microphytobenthos (MPB) and aquatic plants) to consumers such as waterbirds. The presence of PUFAs C18:2ω6 and C18:3ω3 in the tissues of waterbirds, coupled with stable isotope diet evidence provided further evidence of FA transfer from basal resources to consumers but exacerbates the inaccuracy of diet determination. The third hypothesis that there is trophic transfer of FAs from basal resources through the food web to waterbirds was therefore supported. Microphytobenthos, and salt marsh plants appear to be crucial sources of several essential FAs for waterbird consumers in estuarine environments. SFAs C16:0 and C18:0 are considered ubiquitous in the tissues of benthic consumers found in the Kowie Estuary (see **Table 18**, Volkman et al. 1998, Bergamino and Richoux 2014). These benthic consumers acquire C16:0 through their consumption of autochthonous basal resources (detritus, MPB, POM and aquatic plant material) found in the Kowie Estuary (**Table 17,** see Bergamino and Richoux 2014). Marsh plants contained high proportions of C16:1ω7 during winter and summer only (18% in both instances, **Table 18**), while C16:1ω7 and C20:5ω3 were detected as major components in MPB in the lower reaches of the Kowie Estuary (Bergamino and Richoux 2014). These FAs have been characterised as diatom-associated components in marine environments and have subsequently been recognised as trophic biomarkers for MPB (Dunstan et al. 1993, Parrish et al. 2000, Bergamino and Richoux 2014).

Table 6: Seasonal mean proportion (% ± SD) of major FA components in the basal resources of the Kowie Estuary (extracted Bergamino and Richoux 2014)

	detritus				micro-phytobenthos				*S. maritima* (C3)				salt marsh plants (C4)			
	winter	spring	summer	autumn	winter	spring	summer	autumn	winter	spring	summer	autumn	winter	spring	summer	autumn
C14:0	1 (0.3)	9 (0.2)	3 (0.2)	2 (1.7)	6 (1.3)	7 (0.4)	4 (0.4)	5 (0.4)	5 (1.3)	2 (0.1)	5 (1.3)	1 (0.1)	3 (1.7)	2 (0.6)	3 (1.7)	1 (0.2)
C15:0	0	9 (0.3)	1 (0.5)	0	**17 (5.0)**	5 (4.4)	2 (0.1)	1 (0.1)	1 (0.4)	1	1 (0.4)	0	0	0	0	0
C16:0	**31 (14.0)**	**28 (0.7)**	**30 (0.7)**	20 ()	**19 (7.0)**	**15 (0.6)**	**32 (0.3)**	**20 (2.3)**	**12 (11.0)**	**31 (0.6)**	**12 (11.0)**	**21 (0.2)**	**26 (9.9)**	**18 (2.6)**	**26 (9.9)**	**19**
C17:0	1 (0.4)	1 (0.1)	1 (0.1)	0	1 (1.0)	1 (0.8)	0	0	1 (0.6)	1 (0.5)	1 (0.6)	0	1 (0.6)	1 (0.3)	1 (0.6)	0
C18:0	4 (1.7)	7 (1.9)	6 (0.2)	4 (3.3)	3 (3.0)	2 (0.01)	2 (0.1)	1 (0.4)	5 (1.6)	2 (0.1)	5 (1.6)	2 (0.1)	3 (1.0)	2 (0.1)	3 (1.0)	3
SFA	47 (18.5)	62 (4.7)	52 (0.3)	30 (13.2)	54 (13.5)	38 (0.7)	45 (1.6)	34 (1.2)	35 (8.1)	40 (2.3)	35 (8.1)	30 (0.5)	42 (8.2)	25 (3.2)	42 (8.2)	31 (0.2)
C16:1ω7	1 (1.0)	**14 (1.0)**	2 (0.3)	1 (0.1)	**15 (1.0)**	**13 (1.5)**	**10 (0.4)**	**18 (2.9)**	9 (6.3)	**10 (1.5)**	9 (6.3)	1	**18 (8.4)**	1 (0.9)	**18 (8.4)**	0
C17:1ω7	0	2 (0.1)	0	0	5 (3.1)	2 (2.3)	0	0	0	0	0	0	0	0	0	0
C18:1ω9	**12 (2.7)**	4 (1.1)	3 (2.2)	4 (3.8)	2 (0.7)	1	2	1 (0.2)	6 (1.3)	7 (1.1)	6 (1.3)	8 (0.8)	4 (2.4)	5 (3.9)	4 (2.4)	14 (0.2)
C18:1ω7	1 (0.3)	4 (0.2)	6 (0.3)	1 (0.6)	3 (0.7)	1 (0.1)	2 (0.1)	1 (0.2)	6 (3.8)	4 (0.3)	6 (3.8)	2 (0.1)	4 (1.5)	0	4 (1.5)	1
MUFA	14 (2.1)	19 (1.9)	12 (2.2)	6 (4.0)	26 (5.8)	19 (1.4)	18 (1.4)	22 (2.2)	19 (5.4)	26 (2.1)	19 (5.4)	11 (0.3)	29 (5.5)	7 (3.3)	29 (5.5)	15 (0.3)
C18:2ω6	**16 (4.3)**	1 (0.1)	**13 (0.8)**	13 (2.0)	2 (1.6)	1	4	1 (0.1)	6 (1.9)	9 (0.3)	6 (1.9)	**33 (0.3)**	3 (1.3)	**15 (4.2)**	3 (1.3)	**19 (0.3)**
C18:3ω3	**23 (11.4)**	0	**17 (1.2)**	48 (16.6)	1 (0.1)	1 (1.0)	**17 (0.5)**	1 (0.1)	8 (5.3)	**14 (0.1)**	8 (5.3)	**26 (0.4)**	4 (3.2)	**53 (10.4)**	4 (3.2)	**33 (0.2)**
C18:4ω3	0	1 (0.2)	0	32 (2.2)	0	0	1	1 (0.1)	5 (1.9)	1	5 (1.9)	0	1 (0.8)	0	1 (0.8)	0
C20:4ω6	0	2 (0.1)	1 (0.5)	0	2 (0.6)	3	1 (0.1)	1 (0.2)	2 (0.7)	0	2 (0.7)	0	1 (1.5)	0	1 (1.5)	0
C20:5ω3	0	6 (0.5)	1 (0.2)	0	8 (5.1)	**16 (0.2)**	7 (0.2)	**22 (1.4)**	**16 (5.3)**	3 (0.1)	16 (5.3)	0	5 (3.2)	0	5 (3.2)	0
C22:5ω6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C22:5ω3	0	0	0	0	0	2 (1.8)	0	0	0	0	0	0.0	0	0	0	2 (1.6)
C22:6ω3	0	0	4 (0.4)	0	1 (0.5)	0	1 (0.1)	1 (0.7)	3 (1.1)	1	3 (1.1)	0	2 (1.4)	0	2 (1.4)	0
PUFA	39 (16.4)	19 (3.1)	36 (2.5)	63 (15.3)	20 (10.4)	43 (0.8)	37 (0.3)	43 (3.0)	47 (13.5)	34 (1.1)	47 (13.5)	59 (0.5)	29 (12.7)	68 (6.4)	29 (12.7)	53 (0.5)

Table 7: Annual mean proportion (% ± SD) of major FA components in consumers of the Kowie Estuary (extracted from Bergamino and Richoux 2014)

	micro-invertebrates	Myscidacea	Shrimp (*P. peringueyi*)	Crabs	Flathead Mullet (*M. cephalus*)	Striped Mullet (*L. dumerili*)	East coast Sole (*S. bleekeri*)
C14:0	6 (3.7)	4	1 (0.54)	2 (1.0)	**14 (2.2)**	**10 (1.3)**	3 (0.9)
C16:0	**27 (13.3)**	1	**20 (6.2)**	**27 (12.2)**	2 (0.6)	4 (0.1)	2 (1.7)
C17:0	2 (1.0)	**23**	1 (0.8)	3 (2.1)	**40 (2.9)**	**35 (5.4)**	**18 (1.9)**
C15:0	2 (1.6)	1	1 (0.1)	1 (0.5)	0	0	2 (1.7)
C18:0	**10 (6.0)**	9	7 (2.3)	14 (8.7)	6 (2.8)	9 (3.5)	**12 (2.0)**
SFA	47 (24.9)	38	30 (4.3)	47 (22.6)	62 (1.6)	58 (7.4)	37 (6.3)
C16:1ω7	5 (4.0)	4	5 (0.9)	5 (4.7)	0	4 (3.0)	5 (2.2)
C17:1ω7	0	0	1 (0.8)	1 (0.8)	0	0	2 (1.9)
C18:1ω9	5 (4.6)	5	8 (1.4)	5 (3.2)	0	1 (1.0)	7 (2.4)
C18:1ω7	2 (1.9)	4	7 (1.3)	4 (2.6)	0	1 (1.0)	4 (3.3)
MUFA	12 (7.8)	13	21 (3.5)	15 (10.0)	0	6 (2.5)	18 (5.4)
C18:2ω6	1 (0.3)	1	2 (0.8)	3 (2.8)	0	0	1 (0.4)
C18:3ω3	1 (1.2)	1	1 (0.7)	2 (2.4)	0	0	1 (0.5)
C18:4ω3	2 (2.2)	2	1 (0.8)	0	5 (4.6)	2 (0.5)	1 (0.9)
C20:4ω6	1 (1.0)	1	3 (0.6)	2 (2.3)	0	3 (0.4)	4 (0.7)
C20:5ω3	**19 (12.0)**	**19**	**23 (2.4)**	**13 (0.9)**	**15 (3.2)**	**10 (9.1**	7 (3.0)
C22:4ω6	0	0	0	0	0	2 (1.9)	2 (1.2)
C22:5ω3	2 (0.7)	2	1 (0.5)	1 (1.2)	4 (2.5)	5 (1.5)	6 (2.3)
C22:6ω3	**13 (6.4)**	**21**	**14 (2.1)**	9 (3.7)	**12 (2.7)**	**11 (3.9)**	**16 (9.6)**
PUFA	39 (17.2)	47	45 (1.6)	30 (14.1)	36 (1.4)	33 (1.9)	38 (9.4)

The relatively high levels of C16:1ω7 and C20:5ω3 that were detected in the profiles of the benthic consumers of the Kowie Estuary may therefore also indicate the dominance of diatoms at the base of the Kowie Estuary food web (Bergamino and Richoux 2014). C20:5ω3, sourced in MPBs and *S. maritima* constituted notably large percentages of the FA profiles of tissues in all three duck species throughout the study year (**Table 12, 13, 14**), as well as in Ruff tissues during spring and summer. Additionally, PUFAs C20:5ω3 and C22:6ω3 were detected in relatively high proportions in all the benthic fauna of the Kowie estuary (13% - 23%). C18:2ω6 and C18:3ω3, which are sourced in *S. maritima*, were detected in significant proportions in the brachyurans *S. catanata* and *H. obiculare* which also comprised a large proportion of the diet of all three duck species (supported by stable isotope evidence in Chapters 3 and 4).

FA analysis did not provide any clear patterns of dietary resource use amongst the three duck species and Ruff, unlike stable isotope evidence in Chapters 3 and 4, primarily because they feed upon dietary resources from several trophic levels. Karnovsky et al. (2012) advocated that tracing dietary pathways from basal resources to apex consumers becomes more difficult as food chain length increases, especially for birds feeding in aquatic environments. FA analysis of Little Egret tissues however revealed that this species has a narrow diet breadth, primarily feeding on flathead mullet (*M. cephalus*) and striped mullet (*L. dumerili*; see **Table 18**), and highlighted the lack of congruence between these nekton feeding consumers and basal resources, thus making diet determination of Little Egret simpler than invertebrate feeding waterbirds. Similarly, Bergamino and Richoux (2014) found a lack of congruence between the FA profiles of benthic fauna and basal resources along the Kowie Estuary. Fatty acids have been successfully used to distinguish the diet of seabirds in several instances (Käkelä et al. 2005, 2006, 2010). The diet breadth of seabirds in comparison to waterbirds is quite narrow however, whereby seabird species dominantly feed upon squid and pelagic fish species, each with their own distinct FA signatures (Ramos and González-Solís 2012, Karnovsky et al. 2012). Neutral FAs of consumers, which represent storage FAs, have been shown to mirror the FA composition of the diet (e.g. Ruess et al. 2002, Pollierer et al. 2010). Similarly, FA analysis to determine the diet of zooplankton and nekton in estuarine environments has also been met with some success (e.g. Alfaro et al. 2006, Richoux and Froneman 2009). However, because waterbirds in estuarine environments feed upon several food sources across several trophic levels (Polis and Strong 1996, Shaner and Macko 2011) and despite using neutral FA analysis, the accurate determination of their diet through FA analysis becomes difficult. Difficulties in accurate diet determination of waterbirds through FA analysis is evident in this study, whereby clear distinctions in the inter- and intra-seasonal diet of waterbirds becomes near impossible due to the similar FA profiles of primary consumers and basal resources.

Additionally, because Cape Shoveller, Cape Teal, Yellow-Billed Duck and Ruff directly feed in the estuarine sediment and mud for prey items, they may indirectly be ingesting microfauna and biofilm (Bocher et al. 2014), further exacerbating the difficulties experienced with diet determination through FA analysis in this study. I therefore reject the fourth hypothesis that FA analysis can provide useful information on the diet of waterbirds feeding in estuaries.

Dietary routing is the core concept behind FA analysis, based on the fact that it is energetically more efficient to incorporate FAs directly from dietary resources into a consumers' tissues without modification (Pollierer et al. 2010). The transfer of marker FAs along food chains therefore may allow separation of different energy channels (Moore and Hunt 1988), but these have not been investigated here and warrant further investigation. The type of tissue used has been recognised as a key caveat in diet determination of consumers. Adipose tissue is often cited as the most reliable tissue for use in FA analysis, as FAs are predictably deposited in consumer adipose tissue (Karnovsky et al. 2012), but other tissues have been successfully used in other works investigating the diet of marine feeding birds (e.g. Guglielmo et al. 2002, Käkelä et al. 2005, Pierce and Mcwilliams 2005, McCue et al. 2009). In the instance of this study, data provided by FA analysis of multiple tissues have not provided sufficient information upon which solid conclusions on waterbird diet can be drawn. The uncertainties in diet determination through FA analysis in this study may be underpinned by the multiple trophic level feeding behaviour of waterbirds resulting in complex diet compositions. Future studies that focus on the determination of waterbird diet should consider a multiple indicator approach, such as stable isotope data coupled with FA analysis, rather than a single biomarker approach (Karnovsky et al. 2012). Additionally, this study emphasises the need for information pertaining to species-specific metabolism and FA manipulation of waterbirds through feeding experiments (Williams and Buck 2010). Furthermore, this study supports the use of non-destructive sampling of waterbirds, and rather promotes the use of non-destructive sampling of blood and feather samples for isotope analysis.

Chapter 6: Conclusions and future recommendations

6.1 Role of waterbirds in aquatic food webs

A key step in evaluating the importance of birds as consumers in aquatic food webs is to first determine whether they can alter the abundance or behaviour of any of their prey species. Research detailing the role of birds as consumers in aquatic food webs has focussed on the marine environment (Boecklen et al. 2011). Seabirds often congregate in large colonies during the breeding season and provide an ideal opportunity for intensive and detailed sampling of a population (e.g. Hobson et al. 1994, Quillfeldt et al. 2010, Ramos et al. 2011). Seabird consumption of fish in the pelagic marine environment rivals that of global fisheries catches (Brooke 2004, Williams and Buck 2010), making seabirds important components of aquatic food webs. By comparison, there are few estimates of the impact of waterbirds foraging in estuarine environments. Waterbirds congregate in huge numbers in estuaries, particularly in Europe where studies in the Wadden Sea reported numbers as high as 2.7 million individuals at certain times (>547 000 individuals of one species alone – *Caladris alpina*). The impact that these waterbirds have on invertebrate and nekton prey communities is undoubtedly significant but relatively unquantified (Szekely and Bamberger 1992, Moreira 1997, Mendonça et al. 2007, Rosa et al. 2008). Steinmetz et al., (2003) discovered that piscivorous birds can significantly alter the size distribution of five fish species. Likewise, Wanink and Zwarts (2001) revealed that waterbirds often preferentially feed upon large polychaete species in European estuaries. Waterbirds along the Kowie Estuary removed a large proportion of prey biomass (up to 9×10^6 kJ), with invertebrate feeders accounting for approximately 46% of the total energy consumption by waterbirds. Similarly, Kalejta-Summers et al. (2009) found that waterbirds accounted for approximately 49% of the total consumption of invertebrate biomass from Rietvlei, Western Cape. Piscivorous waterbirds however, accounted for the greatest energy consumption in the Kowie Estuary (54% of total consumption), particularly during the summer months (up to 38 kg/month). Likewise, Froneman et al. (2011) revealed that piscivorous birds consumed large quantities of fish along the Riet River, ingesting up to 141 kg of fish in a single month. The full influence of waterbirds on aquatic prey communities along the Kowie Estuary still requires in-depth investigation and quantification. Nevertheless, my study has provided some insight into their foraging behaviour and seasonal diet, which could be used as baseline information for future studies that aim to further quantify the role of waterbirds in estuaries.

Chapter 2 revealed that the Kowie Estuary has the highest Shannon-Weiner diversity index compared to several other South African estuaries, which may be signifying high ecosystem productivity. The total energy consumption of waterbirds in the Kowie Estuary was however low compared to other South African estuaries (see **Table 3**). While numerous attempts have been made to accurately quantify the predatory role that waterbirds play in estuarine ecosystems, there are few studies that have been able to draw meaningful comparisons between waterbirds and other aquatic consumers, such as fish, presumably because it is difficult to accurately quantify the total number of fish within a natural ecosystem. Rosa et al. (2008) discovered through exclusion experiments that waterbirds had a similar deleterious effect to fish consumers on an estuarine invertebrate community (also see Baird et al. 1985, Raffaelli and Milne 1987, Szekely and Bamberger 1992, Bottom et al. 1994, Mendonça et al. 2007). Future studies that aim to quantify the predatory role of waterbirds should consider similar exclusion experiments (but see Kalejta 1993).

6.2 Drivers and implications of diet shifts

Waterbirds along the Kowie Estuary exhibit short and long term diet shifts, yet the drivers of these dietary shifts are relatively unknown. The availability of prey resources along the mudflats of the Kowie estuary most likely underpins why waterbirds may shift their diet (Gawlik 2002). More specifically, waterbirds may exhibit diet shifts to maximise their nutritional intake by focussing their foraging on the most abundant food resources (e.g. Bocher et al. 2014). The flexible foraging behaviour observed in waterbirds is an important mechanism that birds utilise to cope with the unique energetic constraints of each discrete breeding stage and moult (i.e. intrinsic factors) and the seasonal progression of environmental and climatic changes (i.e. extrinsic factors; e.g. Weimerskirch et al. 1993, Charrassin et al. 1998, Cherel et al. 2014). Cape Shoveller, Cape Teal and Yellow-Billed Duck, based on anecdotal evidence, are regarded as generalist feeders (Hockey et al. 2005). Chapter 2 revealed how the populations of waterbirds fluctuated from month to month. In many instances, several waterbird species may be in direct competition with one another for available food resources. In the presence of direct interspecific competition, one species may be required to shift its diet to maximise its nutritional intake (Bolnick et al. 2011, Araújo et al. 2011). One such example may be when Ruff returned to the Kowie Estuary. Prior to the arrival of Ruff, Cape Shoveller were observed dabbling for invertebrates (personal observations 2013).

Upon the arrival of Ruff, the population of Cape Shovellers made a distinct and marked change in their feeding behaviour, from dabbling in the water column to feeding directly on *S. maritima* and salt marsh plants (Personal observations 2013). When Ruff finally returned to Europe in early March 2014, Cape Shoveller reverted back to dabbling for invertebrates within days (Personal observations 2014). Despite these evident changes in the foraging behaviour of Cape Shoveller, the trophic niche of the species appeared to increase in size from winter to spring and summer. The data presented in Chapter 3 provided evidence of the importance of sample size in stable isotope analysis in diet and niche determination. Additionally, the stable isotope results presented in Chapter 3 have also provided some evidence of dietary specialisation on an individual level. While there will inevitably be variation in the isotopic values of tissues amongst individuals of a species, there are instances where Cape Shoveller, Cape Teal and Yellow-Billed Duck individuals are significantly different to other individuals of their species. Competition for food resources is likely a key factor driving dietary variation within and amongst species (see Araújo et al. 2011). Despite a growing body of literature that highlights incidences of conspecific individual specialisation (Bolnick et al. 2002, Martínez del Rio et al. 2009, Vander Zanden et al. 2010, Catry et al. 2014, but also see Bolnick et al. 2003, Araújo et al. 2011 for reviews), the majority of ecological models dealing with predator-prey dynamics, intraspecific competition and food web structure assume that individuals of a single species are identical in their use of available food resources (Bolnick et al. 2003). Semmens et al. (2009) found that the geographical distribution of food resources that several populations of Grey Wolves encountered directly influenced their diet choices. Presumably, waterbirds will attempt to select for the most nutritious food sources available to them considering that flight is a highly energy demanding (Williams and Buck 2010). In the event of new/existing inter- and intraspecific competition for similar resources, a species may switch its diet to primarily feed on the next most nutritious food resource (Williams and Buck 2010, Araújo et al. 2011). Consequently, diet switching may allow waterbirds to maximise their dietary intake of essential FAs and meet their energy demands. Additionally, diet switching of waterbirds with broad diet breadths (i.e. “generalist” feeders) implicates them as regulators of the entire aquatic food web (Steinmetz et al. 2003), as they may feed across several trophic levels (e.g. primary producers and invertebrate consumers; see Cape Shoveller in Chapter 3).

6.3 Stable isotopes and fatty acids as dietary tracers in waterbirds

This thesis has shown that stable isotopes are a useful tool in determining the diets of waterbirds from several tissues. The use of stable isotopes in ecology has advanced greatly over the past five decades, from a novel technique to a ubiquitous tool in the ecologist's toolbox (Forero and Hobson 2003). However, there are certain issues of contention when using stable isotopes to determine the diet of waterbirds. Most evident of them all, is the choice of tissue sampled. Nearly all body tissues of birds have been used to determine the diet of a species with success. Non-destructive sampling of tissues, such as sampling non-primary flight feathers and blood, can be obtained fairly quickly and ethically and is the preferred sampling method for studies on birds. Stable isotope analysis of these tissues have been successfully used to compare the diet of seabirds during the breeding and non-breeding season (Käkelä et al. 2007, Boecklen et al. 2011). The use of feathers and blood tissue in the diet determination of seabirds is fitting, as the dissimilarity amongst the turn-over rates of these tissues has allowed comparisons between long term (i.e. feathers) and short term (i.e. blood) diets to be made with relative ease. But because most waterbirds are considered omnivorous (Hockey and Turpie 1999), the use of one or two body tissue to determine diet may underestimate the diet breadth and time span in which waterbirds in estuaries shift their diet. Nevertheless, the non-destructive sampling of feathers and blood is fully supported as they can still provide information on the complexities of predator-prey relationships between waterbirds and prey communities in estuarine food webs. The separation and analysis of blood into its' constituent components broadens the short-term time scale in which the diet of waterbirds can be quantified (Boecklen et al. 2011, Hobson 2011). Similarly, collection of several feather types, such as non-flight feathers, pectoral feathers and down feathers will additionally allow a broad time-frame of diet to be investigated (Boecklen et al. 2011, Ramos and González-Solís 2012). Additionally, the separation and analysis of varying parts of feathers will provide information on diet over varying time scales (see Grecian et al. 2015). SIAR models are immensely useful in the determination of consumer diets, and have become a staple amongst stable isotope ecologists. However, there are several caveats that require serious consideration when using SIAR models to calculate the diet of waterbirds. As previously mentioned, waterbirds feeding in estuaries feed on numerous food sources, but SIAR models are generally limited to handle only five to seven dietary sources. Scientists need to carefully consider all dietary resources available to waterbirds, and only select those that may be supported by anecdotal evidence or direct observations on foraging (Karnovsky et al. 2012).

There may be instances where the isotopic signature of a waterbird species may be closely associated with a dietary signature that may have no dietary significance. Overall, stable isotopes are an invaluable tool in unravelling the complexities of waterbird diet, as well as aiding scientists to determine the role that waterbird populations play in aquatic food webs. Through the use of stable isotopes, this thesis has revealed that micro-invertebrates and *S. maritima* are important food resources for waterbird species along the Kowie Estuary. Conservation of the components of the Kowie Estuary food web should therefore become imperative, particularly primary producers such as marsh plants, microphytobenthos and *S. maritima*. The pitfalls surrounding the use of FA analysis in waterbird studies are far more serious. A major caveat is the ability of waterbirds to manipulate FA *in vitro* (Williams and Buck 2010). This is a contentious issue however, with evidence that both supports and refutes this claim, but ecologists nevertheless need to consider how waterbirds may metabolise/catabolise FA, and how these processes may influence tissue FA profiles. This study has provided evidence that suggests that FA analysis may not be a reliable tool for the determination of waterbird diet because of the tropic transfer of FA through the food web from basal resources to primary consumers and finally to waterbirds. Additionally, because all three duck species and Ruff forage for prey items in the estuarine sediments, they may be invariably ingesting biofilm, which further exacerbates the complexity of diet determination in waterbirds. FA analysis is a useful tool however in investigating and quantifying the feeding niche of waterbirds (Hebert et al. 2009, Ramírez et al. 2009). Further investigations using feeding experiments are required to understand how FA analysis can be better used in studies on waterbirds.

6.4 Conclusions and future considerations

Previous studies that have calculated the energy and/or prey consumption by waterbirds based on body mass have been heavily criticised, since the use of body mass alone to calculate energy consumption can often lead to gross under- and over-estimates (Moreira 1997, Mendonça et al. 2007). Despite criticisms, basic energy consumption studies have proven useful in determining some measure of the impact that waterbirds play in aquatic food webs (e.g. Master et al. 1993, Heymans and Baird 1995, Mendonça et al. 2007, Kalejta-Summers et al. 2009, Froneman et al. 2011, Terörde and Turpie 2012). Estimates of the energy transfer between consumers and their prey are important to understand the interactions of organisms in an ecosystem and the consumption efficiency of consumers (Wooton 1994, Krivan and Vrkoc 2004).

Energy consumption calculations may not accurately reflect the real functional implications of a trophic link (Mendonça et al. 2007). As such, it is recommended that energy flow calculations should be complementary to traditional ecosystem experiments rather than as an alternative approach (Mendonça et al. 2007). The non-lethal and indirect effects of consumer foraging behaviour is not accounted for in energy consumption calculations (Mendonça et al. 2007, Kalejta-Summers et al. 2009, Froneman et al. 2011). Manipulative *in situ* experiments are therefore the least equivocal way to accurately determine and quantify whether prey communities are significantly affected by waterbirds. Moreover, energy flow and manipulative field experiments should be complementary rather than alternative approaches to the study of the shore as a system (Moreira 1997, Mendonça et al. 2007). In addition, ecosystem experiments need to consider not only the overall deleterious effects of waterbirds of prey populations, but also on how waterbirds can manipulate the size distribution of fish and invertebrate prey communities through selective feeding patterns.

The use of stable isotopes and FAs has allowed scientists to determine the trophic ecology of aquatic food webs in greater detail than ever before, while the application of stable isotopes in ecological research is steadily increasing (Hobson 2011). Waterbirds play roles as vectors of nutrient transfer between aquatic and terrestrial ecosystems. Conservation priorities need to focus on the preservation of all components of the aquatic food web in the Kowie Estuary, but more specifically in the lower trophic levels of the food web. Future studies of waterbirds need to focus attention on the role that they play a vectors linking nutrient transfer between aquatic and terrestrial habitats. Stable isotope and FA biomarkers can further provide information on the fluctuations in top-down pressure that waterbirds exert on prey communities (Steinmetz et al. 2003). In support of non-destructive sampling, museum specimens are an underutilised and reliable source of potential tissue samples to be used in isotopic analysis (Dalerum and Angerbjörn 2005). Of particular interest are species that may be threatened, near extinct or extinct in the wild that could be compared to similar extant, more abundant species (Bond and Jones 2009). Alternatively, museum specimens may provide long-term sources of data (Cherel et al. 2014) on diet shifts that could be associated with climate change and its accompanying ramifications (Greig et al. 2012).

Numerous species of waterbirds currently face a steady decline in their global populations (BirdLife International 2010). Estuaries in South Africa not only play home to numerous resident waterbird species, but also act as non-breeding sites for several dozen migratory species (Hockey et al. 1992, Hockey and Turpie 1999). Additionally, estuaries along South Africa's east coast act as stop-over roosting sites for migrating birds (Turpie et al. 2002).

Conservation priorities therefore need to be focussed on the conservation and correct management of estuarine wetlands (Granadeiro et al. 2007). The correct management and conservation of estuarine wetlands requires accurate information on the number and distribution of waterbirds present within estuaries along South Africa's coastline (Turpie 1995, Turpie et al. 2002, Granadeiro et al. 2007). Because waterbirds generally congregate in high concentrations in only a few feeding sites along an estuary, local persistent impacts on these feeding areas can ultimately have serious negative ramifications on the number and carrying capacity of waterbirds in estuaries (Turpie et al. 2002, Granadeiro et al. 2006, 2007). In the case of the Kowie Estuary, the localised feeding areas used as study sites in this thesis are often subjected to numerous anthropogenic impacts, such as bait collection, general human traffic thoroughfares as well as recreation sports sites. Hence, a good knowledge of the bird distribution is of major importance for conservation planning of the Kowie Estuary and its associated wetlands. Seabirds have been identified as useful indicators of habitat health in marine ecosystems since the early 1980's (reviewed by Piatt et al. 2007). By comparison, there is little information on how waterbird populations can reflect changes in estuarine habitats (e.g. Smit 1981, Kaletjta-Summers et al. 2009, Froneman et al. 2011). Estuaries have been regarded as one of the most biologically diverse ecosystems on Earth, and as such require specific conservation status. Many waterbirds directly depend on estuaries as over-wintering and/or breeding sites (Hockey et al. 1992). Fluctuations in the population size of waterbirds in estuaries may indicate changes in the structure and productivity of estuarine food webs, which in turn may be directly linked to anthropogenic activities.

How adjacent habitats are linked to one another through allochthonous inputs is a growing field of study in aquatic ecology, and the role that waterbirds play in allochthony can be further investigated through the use of stable isotopes and FA analysis. The use of multiple tissue with dissimilar turn-over rates affords ecologists the opportunity to investigate how and when waterbirds may shift their diet over time. Future studies need to consider the predatory pressure exerted by waterbirds more seriously when determining drivers of aquatic community structure (Polis and Strong 1996, Polis et al. 1997b, Steinmetz et al. 2003, Rosa et al. 2008). The effect that waterbirds have on aquatic communities has been recognised since the mid 1970's, when Blaber (1973) discovered that nekton feeding waterbirds significantly reduced the population of a resident fish species in warm temperate estuary in the Eastern Cape. To fully understand the mechanisms of food web structure and functioning, all possible components need to be considered (Polis et al. 1997a, 1997b). Through the application of stable isotope and FA analysis, the true role that waterbirds play in shaping aquatic communities can be unravelled.

But to do so, current Bayesian mixing models, such as SIAR need to allow the incorporation of more dietary sources into statistical tests, while the models need to become more robust to accommodate increased intra- and interspecific variability. The further use of stable isotopes and FA analysis in ecological studies will also allow the theory of "the individual niche" (Bolnick et al. 2003) to be investigated more precisely. This may be particularly relevant for trophic studies of waterbirds, whereby social interactions and hierarchies amongst and within individuals of a species may have a significant role to play in their foraging ecology (Araújo et al. 2011). Investigations into the dietary variations amongst individuals of a species using stable isotopes and FAs may provide information on ecological processes that may otherwise be/have been difficult to observe or quantify. More importantly, accurate determination of individual diets may provide insight into the foraging plasticity of a species which in turn may provide information on the potential for species evolutionary change (Araújo et al. 2011).

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Chapter 8: Appendices.

Table 8: SIAR mean percentage contributions (95% confidence intervals) of potential prey items to seasonal diet composition of selected waterbird species.

Diet Items	winter	spring	summer	autumn
Cape Shoveller				
marsh plants	0	0	76 (33-92)	0
S. maritima	6 (0-15)	37 (17-60)	24 (0-38)	32 (0-13)
micro-invertebrates	50 (18-64)	30 (6-53)	0	47 (35-78)
Mysidacea	20 (0-34)	9 (0-23)	0	6 (0-36)
P. peringueyi	17 (0-40)	13 (0-33)	0	9 (0-32)
crabs	7 (0-27)	11 (0-29)	0	6 (0-15)
Cape Teal				
micro-invertebrates	39 (19-60)	38 (18-59)	23 (0-44)	8 (0-18)
Mysidacea	28 (9-46)	32 (14-50)	31 (3-55)	5 (0-12)
P. peringueyi	20 (0-38)	21 (0-40)	26 (0-50)	14 (0-28)
crabs	13 (0-32)	9 (0-22)	21 (0-38)	73 (60-86)
Yellow-Billed Duck				
micro-invertebrates	10 (1-45)	11 (0-34)	12 (0-33)	34 (2-63)
Mysidacea	72 (51-96)	57 (15-67)	47 (30-82)	16 (0-37)
P. peringueyi	12 (0-25)	22 (0-36)	25 (0-43)	23 (0-46)
crabs	6 (0-32)	10 (0-18)	16 (0-28)	27 (1-49)
Ruff				
micro-invertebrates	0	70 (41-94)	65 (28-88)	0
Mysidacea	0	19 (0-39)	20 (0-40)	0
Isopoda	0	11 (0-30)	15 (0-33)	0
Little Egret				
M. cephalus	87 (65-98)	26 (2-48)	83 (61-92)	43 (9-85)
L. dumerilii	4 (0-14)	19 (0-39)	6 (0-22)	18 (0-40)
S. bleekeri	2 (0-6)	18 (0-38)	2 (0-7)	10 (0-29)
P. peringueyi	3 (0-8)	16 (0-34)	3 (0-9)	12 (0-32)
crabs	4 (0-13)	21 (0-40)	6 (0-21)	17 (0-39)

Table 9: SIAR model mean percentage contributions (95% confidence intervals) of potential prey items to seasonal diet of Cape Shoveller (*Anas smithii*).

	Marsh plants	*S. maritima*	micro-inverts	Mysidacea	*P. peringueyi*	crabs
winter						
flight	79 (55-92)	21 (2-38)	0	0	0	0
down	0	22 (2-39)	32 (7-59)	16 (0-34)	17 (0-7)	13 (0-31)
claw	0	7 (0-16)	40 (17-66)	23 (1-42)	21 (0-41)	9 (0-22)
blood	0	12 (0-25)	40 (18-64)	17 (0-34)	20 (0-40)	11 (0-27)
muscle	0	5 (0-12)	43 (21-64)	30 (12-48)	16 (0-35)	7 (0-18)
liver	0	6 (0-10)	50 (26-74)	20 (0-39)	17 (0-40)	7 (0-20)
spring						
flight	80 (63-94)	20 (0-39)	0	0	0	0
down	0	26 (8-41)	41 (18-62)	11 (0-28)	14 (0-34)	9 (0-26)
claw	0	16 (1-30)	49 (25-70)	12 (0-29)	15 (0-37)	8 (0-22)
blood	0	10 (0-24)	62 (37-82)	10 (0-26)	12 (0-28)	6 (0-16)
muscle	0	12 (1-22)	39 (24-54)	19 (4-34)	20 (1-38)	9 (0-23)
liver	0	37 (17-60)	30 (6-53)	9 (0-23)	13 (0-33)	11 (0-29)
summer						
flight	78 (47-89)	22 (0-38)	0	0	0	0
down	0	39 (18-59)	36 (11-59)	7 (0-20)	10 (0-28)	8 (0-24)
claw	0	27 (11-42)	42 (19-61)	10 (0-25)	13 (0-32)	8 (0-23)
blood	76 (33-94)	24 (0-30)	0	0	0	0
muscle	0	10 (0-12)	47 (36-67)	14 (1-36)	18 (0-32)	6 (0-15)
liver	0	4 (0-9)	58 (0-87)	18 (0-36)	14 (0-38)	6 (0-15)
autumn						
flight	0	65 (53-78)	16 (1-30)	4 (0-12)	7 (0-19)	6 (0-18)
down	0	32 (20-44)	27 (9-44)	10 (0-23)	16 (0-34)	14 (0-31)
claw	0	25 (9-40)	37 (17-58)	12 (0-26)	16 (0-34)	10 (0-27)
blood	0	16 (1-30)	36 (16-59)	16 (0-31)	20 (0-38)	12 (0-29)
muscle	0	5 (0-13)	58 (35-78)	18 (0-36)	14 (0-32)	6 (0-15)
liver	0	32 (16-47)	47 (26-64)	6 (0-18)	9 (0-25)	6 (0-17)

Table 10: SIAR model mean percentage contributions (95% confidence intervals) of potential prey items to seasonal diet composition of Cape Teal (*Anas capensis*).

	Marsh plants	*S. maritima*	micro-inverts	Mysidacea	*P. peringueyi*	crabs
winter						
flight	0	0	25 (1-48)	45 (22-69)	23 (0-44)	8 (0-21)
down	0	0	12 (0-29)	50 (28-71)	24 (0-47)	14 (0-32)
claw	0	0	12 (0-26)	35 (18-52)	31 (5-54)	22 (2-39)
blood	0	0	39 (19-59)	28 (9-45)	20 (0-38)	13 (0-32)
muscle	0	0	50 (29-67)	18 (0-35)	22 (0-42)	10 (0-24)
liver	0	0	59 (36-81)	17 (0-36)	16 (0-36)	8 (0-22)
spring						
flight	0	0	24 (0-45)	45 (22-70)	23 (0-43)	8 (0-21)
down	0	0	17 (0-38)	37 (6-67)	24 (0-47)	23 (0-43)
claw	0	0	22 (0-41)	39 (17-59)	28 (2-50)	11 (0-26)
blood	0	0	38 (18-58)	32 (14-50)	21 (0-39)	9 (0-22)
muscle	0	0	50 (29-70)	23 (3-42)	19 (0-39)	8 (0-21)
liver	0	0	56 (32-79)	17 (0-34)	17 (0-38)	10 0-30)
summer						
flight	44 (27-55)	42 (31-52)	14 (0-30)	0	0	0
down	0	0	44 (24-64)	25 (2-45)	21 (0-42)	10 (0-24)
claw	0	0	42 (9-79)	18 (0-38)	20 (0-44)	20 (0-40)
blood	0	0	23 (0-43)	31 (4-55)	26 (0-50)	21 (0-38)
muscle	0	0	43 (10-76)	15 (0-47)	20 (0-43)	22 (0-42)
liver	0	0	28 (6-47)	14 (0-30)	15 (0-47)	33 (15-51)
autumn						
flight	64 (45-81)	19 (7-32)	17 (0-32)	0	0	0
down	0	0	63 (29-92)	16 (0-41)	14 (0-36)	7 (0-20)
claw	59 (38-75)	24 (10-37)	17 (0-34)	0	0	0
blood	0	0	8 (0-18)	5 (0-12)	14 (0-28)	73 (60-86)
muscle	0	0	47 (14-86)	18 (0-42)	21 (0-44)	14 (0-34)
liver	0	0	48 (19-77)	8 (0-24)	20 (0-45)	24 (2-43)

Table 11: SIAR model mean percentage contributions (95% confidence intervals) of potential prey items to seasonal diet composition of Yellow-Billed Duck (*Anas undulata*).

season	*S. maritima*	*Codium* spp.	micro-inverts	Mysidacea	*P. peringueyi*	crabs
winter						
flight	13 (0-37)	71 (34-99)	16 (0-47)	0	0	0
down	0	0	12 (0-36)	67 (33-94)	14 (0-37)	7 (0-19)
claw	0	0	10 (0-29)	58 25-89)	21 (0-46)	12 (0-31)
blood	0	0	24 (1-45)	40 (15-66)	23 (0-45)	13 (0-33)
muscle	0	0	10 (0-24)	74 (53-92)	11 (0-29)	5 (0-15)
liver	0	0	8 (0-20)	77 (58-93)	11 (0-27)	5 (0-14)
spring						
flight	15 (0-42)	74 (33-98)	11 (0-33)	0	0	0
down	0	0	31 (15-56)	22 (0-43)	24 (0-47)	23 (0-43)
claw	0	0	22 (1-40)	16 (0-34)	27 (0-50)	35 (16-54)
blood	0	0	11 (0-34)	71 (37-96)	12 (0-35)	6 (0-18)
muscle	0	0	35 (13-56)	20 (0-39)	25 (0-48)	20 (0-37)
liver	0	0	23 (0-44)	43 (16-73)	22 (0-45)	11 (0-26)
summer						
flight	0	0	31 (0-56)	38 (9-56)	21 (0-42)	11 (0-28)
down	0	0	29 (0-53)	26 (0-49)	24 (0-47)	21 (0-41)
claw	0	0	12 (0-33)	66 (39-89)	14 (0-34)	8 (0-20)
blood	0	0	12 (0-32)	57 (30-82)	20 (0-42)	12 (0-29)
muscle	0	0	32 (8-53)	38 (19-58)	19 (0-38)	11 (0-28)
liver	0	0	16 (0-36)	64 (42-85)	14 (0-32)	6 (0-17)
autumn						
flight	3 (0-10)	5 (0-14)	92 (81-99)	0	0	0
down	0	0	10 (0-23)	52 (34-70)	28 (3-49)	10 (0-23)
claw	0	0	34 (12-57)	27 (3-46)	26 (1-47)	13 (0-27)
blood	0	0	34 (12-63)	16 (0-37)	23 (0-46)	27 (0-49)
muscle	0	0	46 (29-62)	32 (11-52)	15 (0-32)	7 (0-19)
liver	0	0	33 (16-51)	43 (22-64)	16 (0-33)	8 (0-23)

Table 12: Seasonal mean proportion (% ± SD) of major FAs detected in the tissues of Cape Shoveller (*Anas smithii*).

	winter				spring				summer				autumn			
	adipose	blood	muscle	liver	adipose	blood	muscle	liver	adipose	blood	muscle	liver	adipose	blood	muscle	liver
C14:0	2 (1.6)	4 (1.2)	0	1 (0.6)	0	4 (0.6)	0	2 (0.5)	1 (0.3)	7 (1.3)	1 (0.4)	2 (0.9)	1 (0.1)	3 (1.1)	0	1 (0.4)
Ci15:0	2 (1.3)	0	2 (0.1)	1 (0.4)	1 (0.4)	5 (0.9)	1 (0.4)	0	1 (0.7)	0	1 (0.4)	1 (0.4)	1 (0.5)	4 (1.6)	1 (0.4)	1 (0.8)
C15:0	0	5 (1.6)	2 (0.2)	2 (1.5)	1 (0.1)	1 (0.6)	2 (1.1)	1 (0.4)	0	2 (1.1)	3 (1.0)	2 (0.9)	0	4 (1.7)	2 (1.5)	2 (0.2)
C16:0	20 (1.0)	16 (1.8)	46 (6.1)	25 (8.1)	22 (4.0)	8 (4.4)	18 (2.6)	25 (4.1)	7 (3.1)	18 (6.5)	19 (5.9)	23 (3.8)	15 (3.6)	16 (1.9)	25 (7.3)	26 (6.8)
Ci17:0	2 (0.5)	3 (0.5)	0	1 (0.1)	0	3 (0.6)	1 (0.5)	0	0	6 (1.4)	1 (0.9)	0	4 (1.0)	3 (0.5)	2 (0.4)	1 (0.6)
C17:0	4 (1.6)	3 (0.4)	1 (0.2)	4 (2.4)	1 (0.6)	2 (0.8)	4 (2.2)	6 (3.7)	1 (0.4)	3 (1.4)	1 (0.2)	3 (0.9)	1 (0.3)	3 (0.3)	2 (1.5)	8 (3.6)
C18:0	10 (2)	11 (4.0)	1 (0.2)	1 (0.4)	2 (0.6)	9 (2.8)	6 (3.7)	9 (8.3)	3 (1.5)	7 (1.0)	1 (0.3)	3 (0.8)	5 (1.0)	11 (3.0)	5 (1.9)	1 (0.3)
SFA	40 (7.1)	34 (8.9)	52 (17.0)	42 (5.7)	26 (8.0)	44 (9.1)	32 (6.5)	33 (3.0)	13 (2.4)	34 (8.0)	27 (6.8)	42 (5.9)	27 (5.3)	40 (9.3)	36 (8.8)	43 (5.0)
C16:1ω7	11 (0.7)	3 (1.2)	21 (3.3)	12 (7.6)	9 (4.5)	2 (1.8)	2 (1.0)	4 (2.2)	5 (1.4)	4 (2.8)	6 (0.7)	10 (0.7)	18 (2.4)	8 (2.2)	9 (3.9)	6 (1.8)
C16:1ω5	1 (0.1)	0	1 (0.2)	0	0	0	8 (1.5)	0	0	0	0	0	1 (0.2)	0	0	0
C17:1ω7	3 (1.7)	1 (0.2)	0	1 (-0.5)	0	0	3 (1.4)	4 (3.6)	0	0	0	0	1 (1.0)	0	2 (1.6)	0
C18:1ω9	17 (2)	2 (0.1)	4 (0.9)	9 (4.9)	3 (0.7)	9 (2.4)	11 (4.4)	12 (6.1)	1 (0.5)	5 (1.7)	6 (1.4)	6 (2.3)	1 (1.0)	5 (1.3)	8 (1.8)	11 (9.2)
C18:1ω7	5 (0.8)	2 (0.4)	1 (0.1)	1 (0.7)	1 (0.9)	2 (1.2)	3 (1.0)	3 (1.7)	12 (1.1)	3 (2.7)	2 (0.9)	3 (0.1)	1 (0.3)	3 (0.7)	3 (1.0)	19 (12.1)
MUFA	37 (6.5)	23 (5.3)	27 (8.9)	9 (1.4)	13 (3.6)	24 (4.6)	28 (3.7)	13 (3.6)	19 (5.2)	20 (4.4)	14 (3.0)	12 (2.3)	22 (7.3)	36 (8.0)	21 (4.1)	15 (3.2)
C16:3ω3	1 (0.8)	0	1 (0.6)	0	14 (3.7)	0	1 (0.5)	0	0	0	2 (0.5)	2 (0.3)	0	0	1 (0.8)	0
C16:3ω4	0	0	2 (0.1)	0	0	0	1 (0.6)	0	0	0	1 (0.8)	0	2 (1.9)	0	1 (0.7)	0
C16:4ω3	1 (0.7)	0	0	0	1 (0.9)	0	2 (0.4)	2 (0.6)	0	0	5 (1.4)	4 (0.3)	2 (1.2)	0	0	3 (1.0)
C18:2ω6	0	1 (0.9)	1 (0.4)	10 (7.1)	0	1 (0.8)	3 (2.3)	1 (0.6)	11 (3.3)	1 (1.3)	0	0	0	1 (0.9)	6 (2.3)	3 (0.9)
C18:3ω6	0	6 (0.8)	0	0	0	3 (2.0)	0	1 (0.3)	0	6 (3.5)	0	2 (0.3)	0	5 (0.7)	0	1 (0.9)
C18:3ω3	3 (0.5)	11 (3.8)	3 (1.2)	5 (3.8)	5 (1.8	14 (4.6)	3 (0.9)	2 (1.9)	3 (0.3)	4 (2.1)	5 (1.3)	7 (0.5)	4 (2.2)	11 (3.8)	3 (1.8)	4 (0.6)
C18:4ω3	0	0	0	3 (2.0)	1 (0.8)	16 (2.1)	1 (0.8)	1 (0.7)	0	0	2 (0.5)	2 (0.6)	1 (0.2)	10 (4.1)	2 (1.4)	1 (0.2)
C20:2ω6	1 (0.6)	0	0	0	2 (1.2)	0	1 (0.2)	0	0	0	8 (1.6)	0	2 (1.5)	0	1 (0.5)	0
C20:3ω6	0	3 (0.3)	2 (1.2)	0	1 (0.5)	2 (0.4)	0	0	0	4 (0.5)	6 (1.7)	0	1 (0.4)	2 (0.3)	0	0
C20:4ω6	1 (0.2)	4 (1.9)	0	3 (2.1)	1 (0.4)	3 (1.5)	2 (1.0)	2 (1.1)	1 (0.6)	1 (0.7)	0	0	1 (0.3)	3 (1.9)	4 (1.0)	1 (0.8)
C20:5ω3	2 (0.6)	7 (2.2)	7 (3.2)	5 (3.7)	21 (5.9)	10 (1.7)	17 (3.0)	15 (2.4)	41 (6.3)	20 (2.8)	23 (7.3)	19 (2.7)	24 (2.3)	6 (2.0)	12 (3.1)	4 (1.8)
C22:4ω6	1 (0.5)	2 (0.6)	0	0	1 (0.9)	1 (0.4)	1 (0.2)	0	2 (0.3)	6 (3.2)	2 (1.3)	3 (0.4)	1 (0.9)	1 (0.5)	0	1 (0.6)
C22:4ω3	0	0	0	1 (0.8)	5 (1.3)	0	0	0	0	0	0	0	2	0	0	1 (0.3)
C22:5ω6	0	0	1 (0.3)	0	1 (0.4)	0	0	0	1 (0.6)	0	1 (0.4)	0	1 (0.5)	0	4 (2.5)	1 (0.9)
C22:5ω3	1 (0.4)	1 (0.3)	1 (0.3)	4 (1.6)	1 (0.5)	2 (0.8)	2 (1.3)	2 (0.8)	1 (0.5)	2 (0.9)	1 (0.3)	1 (0.3)	2 (0.6)	0	3 (1.2)	1 (0.5)
C22:6ω3	1 (0.3)	2 (1.3)	1 (0.5)	4 (2.0)	1 (0.4)	1 (0.1)	2 (0.6)	2 (1.1)	1 (0.6)	2 (0.8)	1 (0.3)	1 (0.5)	3 (0.9)	2 (1.3)	2 (0.2)	1 (0.2)
PUFA	11 (0.7)	36 (2.7)	19 (1.7)	37 (3.2)	54 (5.8)	28 (3.6)	36 (4.1)	55 (5.3)	63 (10.3)	41 (4.8)	56 (5.9)	45 (5.2)	47 (5.6)	20 (1.3)	39 (3.1)	42 (3.6)

Table 13: Seasonal mean proportion (% ± SD) of major FAs detected in the tissues of Cape Teal (*Anas capensis*).

	winter				spring				summer				autumn			
	adipose	blood	muscle	liver	adipose	blood	muscle	liver	adipose	blood	muscle	liver	adipose	blood	muscle	liver
C14:0	5 (0.9)	2 (0.9)	4 (0.8)	1 (0.6)	0	4 (0.5)	4 (0.3)	2 (1.2)	3 (0.5)	2 (0.5)	1 (0.8)	1 (0.1)	2 (0.5)	3 (1.4)	6 (0.3)	10 (4.2)
i-C15:0	1 (0.2)	0	2 (0.5)	0	2 (0.9)	2 (0.7)	3 (0.8)	0	1 (0.1)	2 (0.2)	0	0	2 (1.2)	0	0	7 (0.8)
ai-C15:0	2 (0.3)	0	1 (0.5)	0	1 (0.5)	1 (0.8)	2 (0.9)	2 (1.3)	1 (0.2)	2 (0.2)	0	0	0	3 (0.6)	2 (0.1)	0
C15:0	4 (1.4)	1 (0.6)	2 (0.6)	1 (1.0)	1 (0.6)	2 (0.9)	3 (0.5)	2 (1.2)	0	3 (1.7)	1 (0.5)	1 (0.1)	1 (0.5)	5 (0.3)	4 (1.8)	3 (1.4)
C16:0	3 (1.1)	42 (16.7)	47 (7.4)	31 (14.7)	12 (0.8)	27 (3.3)	22 (4.4)	15 (1.9)	3 (0.4)	16 (6.4)	11 (3.5)	1 (0.3)	8 (0.9)	24 (6.0)	22 (6.4)	6 (3.0)
Ci17:0	0	0	0	0	0	0	0	0	1 (0.3)	0	1 (0.6)	1 (0.2)	0	0	0	0
C17:0	1 (0.3)	11 (7)	1 (0.4)	13 (2.2)	0	4 (0.5)	1 (0.3)	2 (1.4)	0	2 (0.3)	7 (1.2)	4 (0.5)	4 (2.2)	6 (0.5)	6 (1.7)	0
C18:0	5 (0.2)	0	4 (1.3)	10 (3.9)	0	1 (0.7)	2 (0.6)	4 (0.4)	2 (0.1)	1 (0.1)	2 (1.0)	2 (0.5)	7 (1.3)	10 (0.8)	3 (0.4)	2 (0.7)
C21:0	1 (0.2)	0	0	0	0	0	0	0	1 (0.4)	4 (0.1)	0	0	0	6 (0.5)	1 (0.3)	0
SFA	21 (1.6)	53 (15.4)	60 (16.8)	57 (11.4)	16 (4.4)	42 (9.3)	37 (7.5)	28 (4.8)	9 (1.1)	29 (5.3)	23 (4.0)	9 (1.4)	25 (3.0)	50 (8.0)	44 (7.5)	28 (3.8)
C16:1ω7	0	4 (2.8)	16 (5.0)	1 (0.7)	5 (0.6)	3 (1.4)	2 (0.9)	3 (1.8)	0	6 (1.8)	0	0	3 (0.3)	0	0	0
C18:1ω9	16 (2.4)	21 (7.0)	4 (1.1)	7 (0.6)	3 (0.8)	3 (1.1)	24 (4.9)	24 (6.4)	2 (0.4)	3 (0.2)	4 (2.6)	6 (0.9)	3 (0.4)	6 (0.2)	4 (1.2)	10 (1.7)
C18:1ω7	6 (1.9)	1 (0.3)	1 (0.5)	3 (1.4)	5 (0.4)	1 (0.5)	3 (1.0)	13 (3.3)	1 (0.1)	2 (0.4)	5 (2.7)	14 (2.3)	3 (0.5)	3 (0.5)	3 (0.5)	6 (1.8)
C20:1ω7	0	0	0	1 (0.8)	0	0	0	0	1 (0.2)	4 (0.7)	0	0	0	0	0	4 (2.1)
MUFA	22 (8.1)	26 (10.9)	22 (8.0)	10 (3.1)	13 (1.2)	8 (1.3)	29 (12.6)	40 (10.3)	3 (0.8)	10 (2.0)	9 (2.5)	19 (6.8)	10 (1.3)	9 (2.9)	(2.3)8	16 (5.0)
C16:3ω4	1 (0.3)	0	2 (0.5)	0	4 (0.4)	2 (0.8)	2 (0.9)	2 (1.1)	2 (0.8)	0	10 (2.9)	0	0	0	0	1 (1.0)
C18:2ω6	0	4 (4.3)	1 (0.9)	6 (3.6)	7 (1.4)	13 (1.4)	3 (0.8)	4 (0.6)	3 (0.9)	2 (0.3)	6 (2.0)	5 (0.8)	2 (0.7)	4 (0.7)	16 (4.8)	0
C18:3ω6	0	0	0	0	0	2 (0.9)	2 (1.1)	3 (1.3)	0	0	0	0	0	0	0	1 (1.0)
C18:3ω3	5 (1.85)	2 (1.1)	3 (0.6)	0	1 (0.4)	0	4 (0.6)	3 (1.0)	4 (0.5)	0	0	0	4 (0.2)	0	0	0
C18:4ω3	3 (0.8)	1 (0.5)	0	0	0	3 (0.5)	0	0	0	0	5 (2.9)	0	0	3 (0.7)	2 (0.3)	0
C20:3ω6	1 (0.3)	0	1 (0.5)	0	5 (0.3)	2 (0.8)	3 (1.0)(	3 (1.1)	0	1 (0.6)	2 (0.8)	5 (0.7)	2 (0.5)	3 (0.5)	3 (1.5)	2 (1.1)
C20:4ω6	0	2 (1.1)	0	8 (3.7)	9 (0.9)	0	0	3 (0.8)	9 (0.2)	0	7 (1.7)	5 (0.5)	0	0	0	1 (1.0)
C20:3ω3	1 (0.1)	1 (0.4)	1 (0.6)	0	8 (2.9)	0	2 (1.1)	0	6 (0.8)	0	0	0	5 (1.8)	0	0	0
C20:4ω3	0	0	0	0	0	11 (0.7)	5 (1.7)	3 (1.8)	0	0	0	0	5 (1.2)	0	0	4 (2.5)
C20:5ω3	0	3 (2.0)	3 (0.7)	4 (2.0)	19 (1.7)	9 (0.7)	8 (0.7)	6	6 (0.2)	29 (1.4)	15 (2.4)	26 (7.8)	21 (2.0)	11 (2.4)	12 (3.1)	22 (13.7)
C21:5ω3	3 (0.4)	0	0	0	6 (0.5)	0	0	0	3 (0.3)	0	0	0	2 (0.7)	0	0	0
C22:4ω6	2 (0.1)	0	0	1 (1.0)	1 (0.4)	2 (0.6)	2 (0.9)	3 (1.0)	8 (0.2)	1 (0.2)	3 (1.9)	5 (0.7)	0	0	3 (0.1)	1 (1.0)
C22:5ω6	6 (1.2)	0	1 (0.4)	1 (1.0)	2 (0.8)	2 (1.0)	2 (0.8)	2 (0.3)	1 (0.1)	1 (0.2)	2 (0.8)	4 (0.6)	2 (0.5)	0	0	4 (2.1)
C22:5ω3	14 (1.0)	2 (0.4)	1 (0.5)	2 (1.2)	1 (0.8)	1 (0.4)	1 (0.4)	1 (0.3)	16 (0.9)	2 (0.7)	1 (1.0)	4 (0.6)	3 (0.4)	2 (0.5)	2 (0.3)	3 (0.5)
C22:6ω3	7 (1.4)	2 (0.5)	1 (0.4)	2 (1.1)	1 (0.5)	1 (0.4)	1 (0.2)	1 (0.2)	19 (1.9)	2 (0.5)	1 (0.2)	2 (1.6)	3 (0.3)	3 (0.6)	2 (0.7)	3 (1.6)
PUFA	43 (4.0)	17 (1.2)	14 (1.0)	25 (2.6)	65 (5.0)	46 (4.3)	32 (2.1)	30 (1.7)	78 (5.9)	39 (7.7)	52 (4.5)	57 (6.8)	50 (5.3)	25 (2.9)	40 (4.8)	41 (5.8)

Table 14: Seasonal mean proportion (% ± SD) of major FAs detected in the tissues of Yellow-Billed Duck (*Anas undulata*).

	winter				spring				summer				autumn			
	adipose	blood	muscle	liver	adipose	blood	muscle	liver	adipose	blood	muscle	liver	adipose	blood	muscle	liver
C14:0	4 (2.1)	3 (1.7)	4 (1.2)	1 (0.5)	2 (0.6)	2 (1.2)	4 (1.4)	1 (0.7)	4 (1.8)	4 (1.2)	4 (0.6)	2 (0.9)	0	5 (0.3)	4 (0.6)	2 (1.4)
i-C15:0	1 (1.0)	0	1 (0.5)	0	2 (0.6)	1 (0.5)	2 (1.0)	1 (1.0)	2 (0.9)	1 (0.4)	0	0	2 (1.0)	3 (0.9)	2 (0.7)	0
ai-C15:0	0	0	0	0	1 (1.0)	1 (0.6)	2 (1.5)	0	2 (0.8)	1 (0.4)	3 (1.2)	0	0	2 (0.9)	1 (0.8)	2 (1.4)
C15:0	1 (0.8)	2 (0.8)	3 (1.3)	1 (0.6)	2 (0.3)	2 (0.8)	0	1 (1.0)	2 (0.5)	0	3 (1.4)	3 (1.0)	4 (1.7)	3 (0.5)	2 (0.9)	2 (1.3)
C16:0	25 (8.6)	2 (0.7)	11 (1.2)	22 (5.2)	5 (4.1)	7 (4.2)	10 (1.8)	13 (3.0)	3 (0.4)	1 (0.5)	7 (1.2)	11 (2.7)	12 (2.8)	23 (4.5)	28 (3.5)	15 (2.2)
Ci17:0	1 (0.9)	2 (0.3)	1 (1.0)	1 (0.5)	2 (1.3)	2 (0.4)	2 (1.0)	2 (1.0)	2 (0.4)	3 (1.2)	4 (1.0)	3 (0.7)	0	0	0	0
C17:0	2 (0.6)	3 (1.7)	3 (1.4)	2 (0.3)	3 (0.7)	3 (1.2)	4 (1.1)	2 (1.1)	1 (0.8)	2 (1.0)	13 (6.8)	3 (0.8)	1 (0.3)	0	3 (0.6)	2 (1.1)
C18:0	10 (1.8)	17 (1.3)	5 (1.0)	16 (2.6)	7 (4.3)	12 (7.9)	2 (1.0)	2 (1.4)	5 (2.8)	12 (6.7)	3 (1.8)	3 (1.3)	1 (0.4)	2 (0.7)	1 (0.8)	4 (0.4)
SFA	43 (8.6)	29 (5.3)	28 (3.3)	44 (8.5)	25 (2.0)	30(4.1)	27 (3.0)	23 (4.1)	21 (1.2)	25 (3.7)	37 (3.7)	26 (3.6)	19 (4.0)	37 (7.5)	42 (9.2)	28 (4.9)
C16:1ω7	2 (1.8)	1 (0.5)	1 (0.5)	2 (1.4)	3 (1.5)	3 (1.7)	1 (1.0)	0	3 (0.5)	1 (0.6)	1 (1.0)	0	6 (2.7)	2 (1.0)	3 (1.5)	3 (1.8)
C18:1ω9	15 (4.3)	4 (1.7)	2 (0.6)	11 (1.6)	14 (3.9)	17 (9.8)	13 (7.0)	4 (0.8)	5 (0.8)	8 (1.7)	4 (1.0)	4 (0.6)	3 (0.9)	25 (5.0)	3 (1.1)	25 (7.4)
C18:1ω7	6 (5.1)	1 (0.3)	2 (0.3)	4 (0.2)	9 (2.1)	9 (5.7)	3 (1.7)	2 (1.0)	6 (0.8)	2 (1.8)	4 (1.9)	4 (0.6)	3 (0.6)	3 (1.1)	1 (0.5)	14 (3.8)
MUFA	23 (6.8)	6 (1.5)	5 (0.6)	18 (4.6)	26 (5.6)	29 (7.1)	17 (6.0)	6 (1.8)	13 (1.4)	11 (3.4)	9 (1.8)	9 (2.5)	12 (1.8)	29 (13.0)	7 (1.2)	41 (11.0)
C18:2ω6	18 (4.5)	15 (8.9)	5 (0.7)	8 (0.9)	2 (0.7)	11 (6.3)	6 (0.7)	8 (2.7)	3 (0.6)	3 (1.1)	9 (0.4)	2 (0.5)	7 (2.1)	3 (0.9)	13 (1.5)	3 (0.7)
C18:3ω6	0	0	0	0	0	0	1 (0.7)	6 (0.7)	0	0	0	6 (0.6)	5 (1.2)	2 (1.1)	2 (1.0)	2 (1.5)
C18:3ω3	0	6 (2.9)	6 (4.1)	1 (0.7)	2 (1.4)	5 (2.4)	7 (5.2)	1 (1.0)	2 (1.2)	7 (1.3)	3 (1.3)	2 (1.4)	1 (0.4)	0	3 (0.6)	2 (1.1)
C20:3ω6	0	0	0	1 (0.4)	2 (0.6)	0	2 (1.3)	1 (0.8)	0	3 (0.7)	3 (1.5)	2 (1.3)	6 (1.6)	2 (1.0)	2 (1.0)	3 (1.2)
C20:4ω6	0	2 (2.0)	0	8 (0.7)	0	1 (0.8)	2 (1.5)	0	0	0	0	2 (1.2)	8 (1.8)	0	0	3 (0.8)
C20:3ω3	0	0	0	0	0	1 (0.4)	0	0	19 (2.1)	2 (1.6)	0	0	8 (1.9)	0	2 (1.4)	0
C20:4ω3	0	11 (7.7)	17 (4.2)	0	0	0	0	0	3 (2.2)	1 (0.7)	0	0	0	5 (1.7)	11 (0.9)	3 (1.9)
C20:5ω3	0	21 (10.4)	25 (9.1)	6 (0.4)	0	5 (4.0)	5 (1.9)	14 (5.8)	7 (1.2)	1 (0.9)	1 (1.0)	16 (2.9)	17 (1.6)	8 (0.7)	9 (0.7)	6 (0.4)
C21:5ω3	1 (1.0)	0	2 (1.6)	0	2 (1.4)	1 (0.4)	0	6 (0.6)	3 (0.8)	2 (1.2)	3 (1.1)	6 (0.3)	6 (1.0)	0	0	0
C22:4ω6	1 (0.8)	0	0	1 (0.8)	3 (0.8)	1 (0.3)	0	7 (2.5)	3 (1.7)	3 (0.9)	2 (0.9)	8 (2.8)	1 (0.2)	2 (1.1)	2 (0.7)	3 (1.1)
C22:5ω6	0	0	3 (1.3)	1 (0.7)	2 (0.8)	1 (0.4)	2 (0.9)	2 (2.0)	1 (1.0)	2 (1.4)	3 (0.9)	2 (0.5)	3 (0.9)	2 (0.9)	2 (1.1)	1 (0.3)
C22:5ω3	1 (0.5)	1 (1.0)	1 (1.0)	2 (0.4)	8 (2.2)	1 (1.0)	2 (0.9)	11 (1.1)	5 (1.0)	12 (4.6)	3 (0.9)	8 (2.4)	2 (0.8)	1 (0.4)	1 (0.4)	1 (1.0)
C22:6ω3	2 (0.9)	1 (0.9)	1 (0.5)	2 (0.3)	10 (3.0)	1 (0.5)	3 (0.7)	13 (1.4)	6 (1.6)	15 (4.8)	4 (1.0)	4 (0.3)	1 (0.3)	1 (0.2)	1 (0.4)	1 (1.0)
PUFA	25 (4.4)	59 (6.4)	61 (7.0)	31 (2.7)	32 (2.7)	29 (2.9)	47 (4.5)	69 (5.1)	52 (4.6)	54 (4.4)	35 (2.4)	58 (4.5)	63 (4.6)	32 (2.1)	50 (4.1)	31 (1.6)

Table 15: Seasonal mean proportion (% ± SD) of major FAs detected in the tissues of Little Egret (*Egretta garzetta*).

	winter				spring				summer				autumn			
	adipose	blood	muscle	liver	adipose	blood	muscle	liver	adipose	blood	muscle	liver	adipose	blood	muscle	liver
C14:0	2 (1.0)	1 (1.2)	2 (0.1)	4 (0.3)	2 (1.2)	4 (0.4)	1 (0.7)	4 (0.3)	2 (1.2)	4 (0.5)	2 (0.4)	3 (0.2)	1 (0.6)	1 (0.3)	3 (0.3)	2 (0.2)
C15:0	2 (0.1)	2 (1.3)	0	1 (0.5)	1 (0.4)	3 (0.6)	2 (1.2)	1 (0.3)	1 (0.5)	3 (0.8)	2 (0.8)	1 (0.3)	2 (0.8)	2 (0.4)	3 (0.4)	3 (0.3)
C16:0	3 (1.2)	2 (1.6)	1 (0.1)	2 (0.5)	20 (12.3)	9 (0.5)	12 (5.2)	6 (1.1)	2 (0.8)	1 (0.2)	10 (4.2)	5 (0.9)	2 (0.8)	10 (1.2)	10 (1.2)	11 (1.2)
Ci17:0	1 (0.6)	0	1 (0.2)	1 (0.4)	1 (0.9)	1 (1.4)	1 (0.6)	2 (0.1)	1 (0.7)	1 (1.0)	1 (0.6)	2 (0.1)	2 (1.0)	2 (0.7)	3 (0.7)	3 (0.7)
Cai17:0	1 (0.5)	0	1 (0.1)	2 (0.5)	0	0	0	2 (0.2)	0	0	1 (0.7)	2 (0.2)	0	1 (0.6)	1 (0.6)	0
C17:0	14 (5.9)	11 (7.1)	0	17 (1.6)	3 (1.5)	3 (0.9)	1 (0.5)	3 (0.2)	3 (1.6)	3 (0.9)	2 (0.4)	10 (0.7)	13 (2.2)	11 (2.5)	10 (1.1)	11 (1.1)
C18:0	1 (1.0)	3 (3.6)	6 (5.4)	2 (0.5)	9 (3.4)	9 (0.6)	1 (1.0)	18 (3.2)	12 (7.3)	8 (0.9)	1 (1.2)	2 (0.2)	5 (0.6)	2 (0.5)	3 (0.5)	2 (0.5)
SFA	24	29	11	29	36	29	28	35	21	20	19	25	25	28	33	30
C16:1ω7	20 (12.1)	1 (1.1)	1 (0.5)	0	21 (13.2)	2 (2.0)	1 (0.8)	1 (0.3)	0	2 (1.6)	1 (1.0)	1 (0.2)	0	0	0	0
C18:1ω9	1 (2.6)	7 (0.7)	0	2 (03)	2 (1.7)	6 (0.3)	0	3 (0.2)	11 (6.6)	7 (2.8)	2 (0.7)	14 (0.8)	5 (0.1)	4 (0.1)	4 (0.6)	5 (0.1)
C18:1ω7	8 (2.5)	5 (1.1)	1 (0.8)	3 (0.4)	8 (3.4)	4 (0.8)	0	3 (0.5)	7 (3.7)	4 (0.5)	3 (0.6)	3 (0.1)	4 (0.3)	4 (0.4)	4 (1.4)	4 (0.3)
MUFA	29	13	2	5	31	12	1	7	18	13	6	18	9	8	8	9
C18:2ω6	8 (4.1)	6 (4.8)	7 (0.1)	12 (1.0)	7 (2.2)	6 (0.4)	1 (0.4)	12 (1.1)	24 (7.8)	5 (0.5)	22 (1.3)	15 (2.3)	3 (0.1)	8 (1.8)	8 (1.5)	8 (0.5)
C18:3ω4	0	0	0	0	2 (1.0)	0	0	0	19 (10.4)	0	0	4 (0.5)	0	0	0	0
C18:3ω3	1 (0.8)	0	0	3 (0.3)	1 (0.7)	0	0	4 (0.2)	1 (0.6)	0	0	3 (0.1)	0	1 (1.0)	1 (1.1)	0
C20:4ω6	7 (5.0)	10 (3.5)	0	3 (0.2)	4 (3.7)	7 (1.0)	6 (0.6)	3 (0.7)	3 (1.9)	7 (0.9)	0	3 (0.5)	9 (0.4)	5 (0.6)	5 (0.5)	4 (0.2)
C20:5ω3	18 (0.7)	15 (4.2)	37 (4.1)	23 (2.5)	14 (7.1)	8 (3.5)	2 (1.4)	17 (1.3)	0	5 (0.1)	0	2 (0.2)	17 (3.8)	21 (3.0)	18 (4.0)	14 (2.3)
C22:5ω3	2 (1.0)	3 (0.4)	2 (1.5)	2 (0.5)	1 (0.5)	3 (0.2)	0	2 (0.2)	6 (1.6)	3 (0.3)	9 (1.6)	9 (0.5)	3 (0.5)	14 (4.6)	4 (0.5)	4 (0.3)
C22:6ω3	10 (6.4)	14 (3.6)	17 (5.3)	17 (1.8)	2 (1.4)	3 (0.9)	0	2 (0.2)	6 (2.3)	3 (0.8)	8 (0.9)	5 (1.4)	8 (1.0)	10 (3.2)	3 (0.2)	3 (0.1)
PUFA	46	47	63	60	31	27	9	40	59	23	39	41	40	59	39	33

Table 16: Seasonal mean proportion (% ± SD) of major FAs detected in the tissues of Ruff (*Phylomachus pugnax*).

	spring				summer			
	adipose	blood	muscle	liver	adipose	blood	muscle	liver
C14:0	5 (3.1)	3 (1.5)	2 (0.8)	4 (0.8)	6 (0.2)	4 (1.4)	4 (0.8)	3 (0.2)
C15:0	2 (0.6)	1 (0.5)	1 (0.4)	5 (3.0)	2 (0.2)	1 (0.5)	1 (0.7)	3 (1.0)
C16:0	18 (11.3)	16 (4.0)	35 (11.7)	18 (6.2)	39 (3.5)	23 (9.2)	15 (6.5)	15 (7.2)
C17:0	3 (1.1)	2 (0.4)	2 (0.5)	1 (1.0)	1 (0.2)	6 (7.0)	2 (0.8)	4 (0.8)
SFA	28	22	40	28	48	34	22	25
C16:1ω7	17 (9.0)	1 (1.0)	1 (1.0)	9 (2.4	0 (0.1)	0	5 (4.0)	1 (1.0)
C18:1ω9	10 (1.5)	11 (2.5)	9 (3.0)	6 (4.2)	10 (0.2)	13 (4.8)	13 (4.4)	9 (4.2)
C18:1ω7	4 (1.1)	4 (1.5)	5 (4.0)	1 (1.0)	3 (0.3)	3 (1.5)	2 (0.1)	5 (2.3)
MUFA	31	16	15	16	13	16	20	14
C18:2ω6	1 (0.7)	12 (5.4)	3 (3.0)	11 (4.8)	3 (0.2)	7 (6.3)	13 (2.8)	6 (3.0)
C18:3ω3	2 (1.2)	4 (3..8)	1 (0.7)	5(4.0)	4 (0.2)	4 (3.7)	4 (3.3)	3 (2.1)
C18:4ω3	3 (2.0)	0	0	0	0	0	0	1 (1.0)
C20:4ω6	0	4 (2.0)	1 (1.0)	1 (1.0)	0	0	5 (4.0)	0
C20:5ω3	10 (0.3)	19 (5.2)	24 (10.4)	22 (7.6)	10 (0.6)	27 (12.3)	22 (10.3)	9 (3.9)
C22:5ω3	2 (1.0)	7 (2.0)	4 (2.0)	2 (0.7)	4 (0.2)	2 (1.7)	2 (0.5)	4 (2.1)
C22:6ω3	1 (1.0)	6 (1.4)	2 (1.6)	3 (2.2)	3 (0.3)	1 (1.0)	2 (0.5)	4 (2.3)
PUFA	19	52	35	44	24	41	48	27